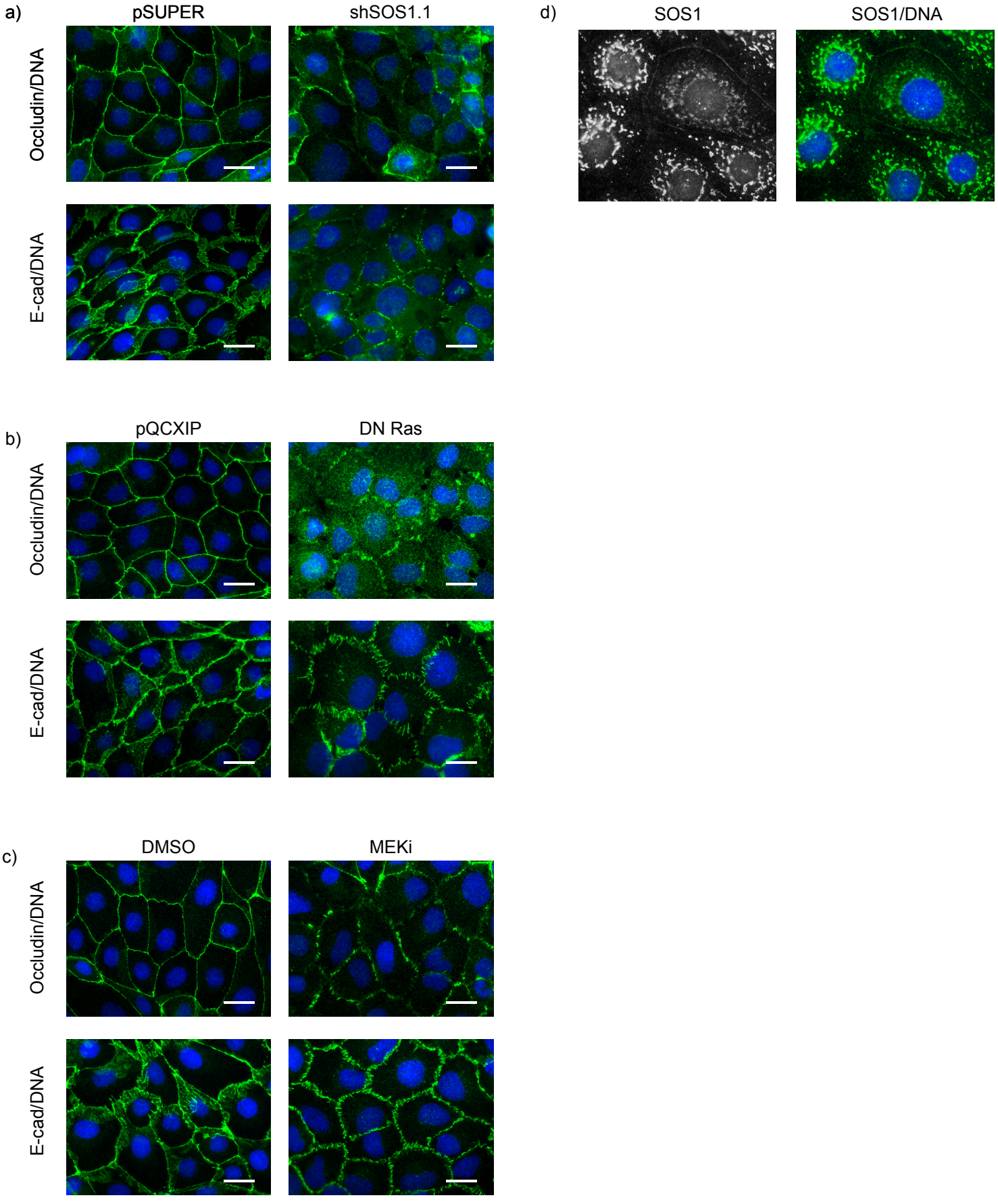
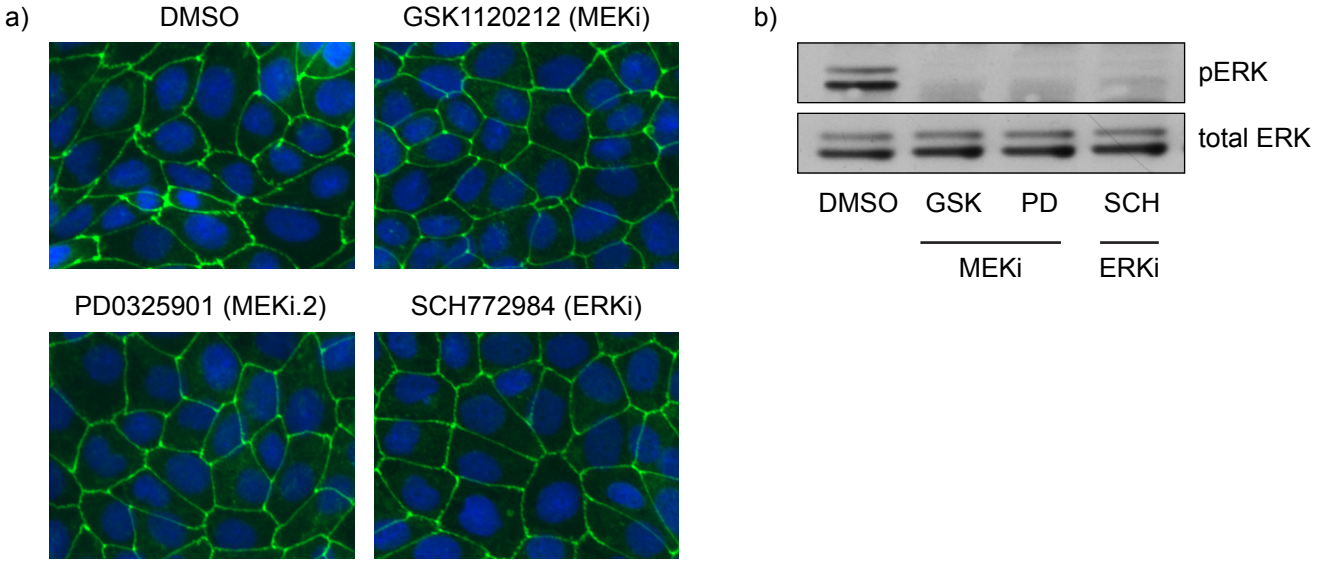


Supplementary Figure 1 SOS1, Ras, MEK and ERK are required for bronchial junction formation. 16HBE cells were stably infected with: (a) pSUPER control or shSOS1.1, (b) pQCXIP control or dominant negative myc-H Ras N17, or (c) Treated with DMSO or GSK1120212 (500nM) for 4 days. Cells were fixed and stained for occludin (tight junctions), E-cadherin (adherens junctions) and DNA (nuclei). Scale bars represent 20μm. d) 16HBE cells were fixed and stained for SOS1 and DNA. In a subset of cells, a junctional signal was detected as shown.



Supplementary Figure 2 MEK and ERK are not required for junctional maintenance in bronchial epithelia. 16HBE cells were seeded on glass coverslips and incubated for 4 days to allow apical junctions to form and mature. The cells were then treated with DMSO, GSK1120212 (500nM), PD0325901 (500nM), or SCH772984 (1μM), and incubated for a further 4 days. a) Cells were fixed and stained for ZO-1 (tight junctions) and DNA (nuclei). No obvious difference is detected upon MEK/ERK inhibition. b) Cell lysates were analysed by western blotting for p-ERK and total ERK to confirm efficient pathway inhibition under these conditions.

Supplementary Figure 3 Ras, MEK and ERK microarray data.

a) Genes downregulated by >1.6-fold versus control, by DN HRas, MEK inhibition and/or ERK inhibition, with an unadjusted p-value <0.05.

Overlapping hits (33): DN Ras, GSK, ERKi (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

column	gene	p-value (attrib	p-value (DN R	Ratio (DN Ras	**Fold change DN Ras**	Fold change D	p-value (GSK)	Ratio (GSK)	**Fold change (GSK)**	Fold change G	p-value (ERKi)	Ratio (ERKi)	**Fold change (ERKi)**	Fold change E	F (Attribute)	SS (Attribute)	SS (Error)	F (Error)
461	ADORA2B	1.95E-05	0.000168	1.83799	1.83799	Control up vs	2.30E-06	2.98912	2.98912	Control up vs	0.00021658	1.79769	1.79769	Control up vs	47.3195	3.76048	0.21192	1
2871	C6ORF15	2.99E-06	8.40E-06	3.75021	3.75021	Control up vs	4.54E-07	6.94665	6.94665	Control up vs	0.00029927	2.22812	2.22812	Control up vs	77.3954	12.6287	0.435122	1
3544	CCND2	0.00035755	0.00083051	2.16843	2.16843	Control up vs	5.14E-05	3.209	3.209	Control up vs	0.00628362	1.72899	1.72899	Control up vs	21.3411	4.44221	0.555074	1
4499	CRABP2	4.07E-06	1.84E-05	1.85724	1.85724	Control up vs	7.30E-07	2.58336	2.58336	Control up vs	4.04E-06	2.13542	2.13542	Control up vs	71.43	3.15993	0.117968	1
4508	CRCT1	0.00025452	0.00018717	1.94461	1.94461	Control up vs	5.01E-05	2.23101	2.23101	Control up vs	0.00123506	1.64583	1.64583	Control up vs	23.4965	2.30055	0.261094	1
5210	DHCR7	0.00052052	0.00024191	1.92423	1.92423	Control up vs	0.00017684	1.98434	1.98434	Control up vs	0.00051709	1.79304	1.79304	Control up vs	19.1629	1.95938	0.272662	1
5628	DUSP5	3.08E-06	3.89E-06	2.10316	2.10316	Control up vs	5.66E-07	2.60258	2.60258	Control up vs	6.31E-06	2.00814	2.00814	Control up vs	76.7606	3.22895	0.112174	1
5790	EGR1	1.55E-07	1.63E-07	3.44941	3.44941	Control up vs	6.94E-08	3.97806	3.97806	Control up vs	6.56E-08	4.01756	4.01756	Control up vs	165.403	8.45669	0.136341	1
5904	ELOVL6	1.52E-06	3.28E-07	1.82707	1.82707	Control up vs	1.57E-06	1.63588	1.63588	Control up vs	2.20E-06	1.60111	1.60111	Control up vs	92.2057	1.33807	0.038698	1
5928	EMP1	0.00034633	0.00161761	2.7376	2.7376	Control up vs	5.47E-05	5.33672	5.33672	Control up vs	0.0328649	1.744	1.744	Control up vs	21.5357	9.40935	1.16512	1
6163	ERRFI1	8.79E-05	0.00026203	1.90287	1.90287	Control up vs	1.17E-05	2.70656	2.70656	Control up vs	0.00125779	1.65687	1.65687	Control up vs	31.5497	3.19131	0.269738	1
6201	ETV4	9.48E-06	5.47E-06	1.66899	1.66899	Control up vs	3.30E-06	1.7294	1.7294	Control up vs	8.57E-06	1.62013	1.62013	Control up vs	57.2483	1.2514	0.0582909	1
6876	FGFBP1	1.26E-05	1.02E-05	5.25917	5.25917	Control up vs	2.66E-06	7.27149	7.27149	Control up vs	0.00038742	2.69931	2.69931	Control up vs	53.0789	14.3763	0.722259	1
7391	FOSL1	2.25E-06	2.29E-06	2.91715	2.91715	Control up vs	4.10E-07	3.8147	3.8147	Control up vs	7.81E-06	2.48401	2.48401	Control up vs	83.3342	6.31973	0.202229	1
7524	FUCA1	2.39E-07	3.68E-06	1.97611	1.97611	Control up vs	2.80E-08	3.59034	3.59034	Control up vs	1.75E-06	2.11901	2.11901	Control up vs	148.124	5.15333	0.0927749	1
7989	GNE	0.00500095	0.00135933	1.92937	1.92937	Control up vs	0.00227296	1.82818	1.82818	Control up vs	0.00711314	1.63455	1.63455	Control up vs	9.59587	1.68635	0.468632	1
12230	HSD3B1	6.55E-08	4.72E-08	2.53472	2.53472	Control up vs	1.62E-08	2.90263	2.90263	Control up vs	1.16E-07	2.29162	2.29162	Control up vs	205.863	4.32821	0.0560659	1
12533	IL1R2	1.93E-05	1.81E-05	3.29224	3.29224	Control up vs	3.72E-06	4.36054	4.36054	Control up vs	0.00050276	2.09709	2.09709	Control up vs	47.4382	7.73501	0.434812	1
13429	KRT18P28	0.00023561	0.00015493	1.91685	1.91685	Control up vs	4.94E-05	2.14975	2.14975	Control up vs	0.00095577	1.63913	1.63913	Control up vs	24.0122	2.13009	0.236556	1
13695	LDLR	1.45E-05	4.44E-06	2.22677	2.22677	Control up vs	5.73E-06	2.1683	2.1683	Control up vs	0.00012922	1.65535	1.65535	Control up vs	51.1626	2.58415	0.134689	1
13862	LMTK3	7.14E-06	3.49E-06	2.49256	2.49256	Control up vs	1.93E-06	2.68369	2.68369	Control up vs	2.24E-05	2.03761	2.03761	Control up vs	61.6678	3.8046	0.16452	1
16621	LOC340598	0.00241572	0.00139445	1.69097	1.69097	Control up vs	0.0006371	1.813	1.813	Control up vs	0.00250649	1.61001	1.61001	Control up vs	12.1074	1.37075	0.301909	1
17152	LOC399965	0.00036044	0.00080086	1.80438	1.80438	Control up vs	7.78E-05	2.30247	2.30247	Control up vs	0.00024569	2.02677	2.02677	Control up vs	21.2922	2.54781	0.319092	1
23939	LYRM1	1.22E-05	1.07E-05	2.04006	2.04006	Control up vs	2.26E-06	2.40156	2.40156	Control up vs	9.61E-05	1.69305	1.69305	Control up vs	53.5374	2.71111	0.135039	1
24517	MIA	1.97E-06	8.15E-07	2.20012	2.20012	Control up vs	8.71E-07	2.18538	2.18538	Control up vs	2.10E-06	2.00782	2.00782	Control up vs	86.164	2.70747	0.0837929	1
25553	MVD	0.00075669	0.00020062	1.957	1.957	Control up vs	0.00059822	1.76793	1.76793	Control up vs	0.0005955	1.76864	1.76864	Control up vs	17.1868	1.7502	0.271558	1
25586	MYEOV	8.03E-06	2.86E-05	3.52848	3.52848	Control up vs	1.37E-06	6.63675	6.63675	Control up vs	0.00257477	1.89845	1.89845	Control up vs	59.8116	12.3828	0.552083	1
26313	NT5E	2.06E-05	9.85E-06	2.88836	2.88836	Control up vs	5.96E-06	3.10909	3.10909	Control up vs	0.00055582	1.81857	1.81857	Control up vs	46.6396	5.11467	0.292437	1
27545	PHLDA1	6.88E-08	1.59E-07	2.21049	2.21049	Control up vs	9.77E-09	3.10095	3.10095	Control up vs	3.38E-07	2.05484	2.05484	Control up vs	203.279	4.24269	0.0556568	1
27733	PLAT	6.34E-06	1.17E-05	1.61702	1.61702	Control up vs	1.34E-06	1.89667	1.89667	Control up vs	5.73E-06	1.69671	1.69671	Control up vs	63.6099	1.49917	0.0628484	1
29855	SCNN1A	1.75E-05	6.58E-05	2.03279	2.03279	Control up vs	2.66E-06	2.98953	2.98953	Control up vs	2.99E-05	2.20682	2.20682	Control up vs	48.7032	4.02241	0.22024	1
31678	SULT2B1	5.34E-06	2.13E-06	1.90606	1.90606	Control up vs	1.65E-06	1.94823	1.94823	Control up vs	2.18E-05	1.60452	1.60452	Control up vs	66.5449	1.79866	0.0720779	1
32149	TGM2	1.97E-08	1.08E-07	4.01643	4.01643	Control up vs	2.37E-09	9.55547	9.55547	Control up vs	2.52E-07	3.48124	3.48124	Control up vs	278.827	16.1955	0.154892	1

Overlapping hits (60): DN Ras and GSK only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

385	ADAM8	0.00012597	0.00037927	2.38378	2.38378	Control up vs	5.38E-05	3.16506	3.16506	Control up vs	0.407428	1.13843	1.13843	Control up vs	28.5824	5.88667	0.549211	1
528	AGPAT9	0.00682359	0.00553383	2.05461	2.05461	Control up vs	0.00238354	2.30811	2.30811	Control up vs	0.275731	1.25102	1.25102	Control up vs	8.65409	2.97076	0.915407	1
844	ANO1	0.000502	0.00045679	1.62309	1.62309	Control up vs	0.00015388	1.76673	1.76673	Control up vs	0.0649154	1.19945	1.19945	Control up vs	19.3642	1.31159	0.18062	1
1320	ATP2C2	1.92E-05	4.00E-05	1.71435	1.71435	Control up vs	9.53E-06	1.92606	1.92606	Control up vs	0.215306	1.09372	1.09372	Control up vs	47.5384	1.97319	0.110686	1
1488	BAIAP2L2	2.78E-05	1.21E-05	1.61796	1.61796	Control up vs	1.17E-05	1.62174	1.62174	Control up vs	0.00317479	1.23346	1.23346	Control up vs	43.0508	1.02692	0.0636099	1
2340	C1ORF116	0.00706544	0.00260698	1.91988	1.91988	Control up vs	0.00514331	1.78247	1.78247	Control up vs	0.270147	1.19673	1.19673	Control up vs	8.55299	1.84112	0.574028	1
2794	C4ORF49	2.30E-07	3.64E-07	1.93988	1.93988	Control up vs	6.54E-08	2.28324	2.28324	Control up vs	0.00057631	1.27249	1.27249	Control up vs	149.547	2.69258	0.0480132	1
3258	CARD11	0.00040484	0.00019024	1.80747	1.80747	Control up vs	0.00011997	1.88282	1.88282	Control up vs	0.00839473	1.37283	1.37283	Control up vs	20.598	1.6052	0.207813	1
3543	CCND1	4.22E-05	0.00035441	2.22702	2.22702	Control up vs	8.16E-06	3.89605	3.89605	Control up vs	0.0474756	1.37227	1.37227	Control up vs	38.4899	6.59824	0.457141	1
3717	CDC42EP5	0.00030045	0.00012222	2.16052	2.16052	Control up vs	0.00044686	1.88944	1.88944	Control up vs	0.226296	1.15714	1.15714	Control up vs	22.4216	2.60358	0.309652	1
3755	CDH4	0.0003967	0.00041776	1.98378	1.98378	Control up vs	7.66E-05	2.4045	2.4045	Control up vs	0.0106331	1.48176	1.48176	Control up vs	20.718	2.73192	0.351632	1
4030	CHRNB1	0.00073459	0.00012565	1.92063	1.92063	Control up vs	0.00074338	1.64932	1.64932	Control up vs	0.00428777	1.4525	1.4525	Control up vs	17.3367	1.45652	0.224036	1
4134	CLDN3	0.00035757	9.37E-05	1.86209	1.86209	Control up vs	0.00018479	1.75734	1.75734	Control up vs	0.00066364	1.59237	1.59237	Control up vs	21.3408	1.49605	0.18694	1
4490	CPVL	1.33E-05	7.73E-06	1.79777	1.79777	Control up vs	3.07E-06	1.94081	1.94081	Control up vs	0.00012897	1.48831	1.48831	Control up vs	52.3581	1.64515	0.0837895	1
5765	EFHD2	0.00185221	0.00407361	1.73523	1.73523	Control up vs	0.00034279	2.27979	2.27979	Control up vs	0.0850547	1.31284	1.31284	Control up vs	13.1436	2.36316	0.479455	1
7536	FUT3	0.00299154	0.00257499	2.02163	2.02163	Control up vs	0.00111155	2.24613	2.24613	Control up vs	0.236689	1.23224	1.23224	Control up vs	11.3218	2.82631	0.665694	1
7768	GDF15	1.84E-06	4.63E-07	2.23135	2.23135	Control up vs	9.96E-07	2.06814	2.06814	Control up vs	3.41E-05	1.57396	1.57396	Control up vs	87.7407	2.46655	0.074965	1
7871	GJB2	0.0147181	0.0131818	1.70488	1.70488	Control up vs	0.00539755	1.88857	1.88857	Control up vs	0.437533	1.14738	1.14738	Control up vs	6.61342	1.75369	0.707122	1
8484	HBEGF	0.00059578	0.00053858	2.28158	2.28158	Control up vs	0.00109964	2.09061	2.09061	Control up vs	0.971436	1.0055	1.0055	Control up vs	18.4282	3.80749	0.550964	1
8587	HES2	5.01E-05	5.48E-05	1.90102	1.90102	Control up vs	1.81E-05	2.1141	2.1141	Control up vs	0.0395811	1.22575	1.22575	Control up vs	36.7652	2.36578	0.171596	1
8767	HMGA1	0.00096079	0.00036953	1.63792	1.63792	Control up vs	0.00033592	1.64962	1.64962	Control up vs	0.0012449	1.50431	1.50431	Control up vs	16.0185	1.05612	0.175816	1
12524	IL1A	0.0001333	0.00029216	1.77976	1.77976	Control up vs	1.93E-05	2.33253	2.33253	Control up vs	0.00273367	1.49757	1.49757	Control up vs	28.1395	2.36035	0.223681	1
12549	IL23A	1.18E-05	8.70E-06	1.68222	1.68222	Control up vs	3.88E-06	1.78445	1.78445	Control up vs	0.00347698	1.23803	1.23803	Control up vs	54.0199	1.37778	0.0680133	1
12572	IL4R	5.55E-05	9.45E-05	1.79834	1.79834	Control up vs	1.11E-05	2.19997	2.19997	Control up vs	0.00843997	1.32808	1.32808	Control up vs	35.7471	2.23823	0.166968	1
12759	ITGA2	0.00015337	0.00028843	2.20823	2.20823	Control up vs	3.05E-05	2.97735	2.97735	Control up vs	0.0204756	1.45349	1.45349	Control up vs	27.0683	4.27125	0.420787	1
13371	KLK6	0.00051929	9.01E-05	2.45761	2.45761	Control up vs	0.00056806	1.98471	1.98471	Control up vs	0.00646018	1.57564	1.57564	Control up vs	19.176	2.78126	0.386769	1
13372	KLK7	0.00097588	0.00046455	3.62066	3.62066	Control up vs	0.00057532	3.47354	3.47354	Control up vs	0.127211	1.47026	1.47026	Control up vs	15.9446	7.66085	1.28124	1
13451	KRT34	0.00093511	0.0479248	1.91976	1.91976	Control up vs	0.0381529	1.99982	1.99982	Control up vs	0.00693281	0.365058	-2.73929	Control down	16.1476	11.818	1.95167	1
13480	KRT86	3.19E-05	2.67E-05	1.88626	1.88626	Control up vs	6.04E-06	2.17192	2.17192	Control up vs	0.00033625	1.55597	1.55597	Control up vs	41.505	2.13535	0.137195	1
13821	LIPG	3.00E-06	1.29E-06	1.66722	1.66722	Control up vs	8.60E-07	1.71425	1.71425	Control up vs	3.09E-05	1.39729	1.39729	Control up vs	77.3458	1.15118	0.0396895	1
15964	LOC10013437	0.0017295	0.00353242	1.86125	1.86125	Control up vs	0.00027772	2.5459	2.5459	Control up vs	0.0369437	1.4632	1.4632	Control up vs	13.4223	2.91422	0.578979	1
23510	LOC731042	0.00046419	0.00034508	1.85819	1.85819	Control up vs	0.00042424	1.82321	1.82321	Control up vs	0.407628	1.09541	1.09541	Control up vs	19.8058	2.01706	0.271579	1
23769	LRP8	6.56E-06	5.75E-06	1.71765	1.71765	Control up vs	1.51E-06	1.90653	1.90653	Control up vs	0.00056978	1.32677	1.32677	Control up vs	63.0685	1.55784	0.0658686	1
23784	LRRC20	6.51E-05	6.88E-05	1.71979	1.71979	Control up vs	1.56E-05	1.94521	1.94521	Control up vs	0.00844166	1.28485	1.28485	Control up vs	34.2359	1.67295	0.130307	1
23909	LY6E	9.27E-05	3.85E-05	1.67577	1.67577	Control up vs	4.72E-05	1.65177	1.65177	Control up vs	0.0136213	1.22099	1.22099	Control up vs	31.0929	1.17081	0.100414	1
23981	MAFF	4.18E-07	4.06E-07	1.73556	1.73556	Control up vs	7.47E-08	1.98371	1.98371	Control up vs	2.98E-06	1.52975	1.52975	Control up vs	128.459	1.64717	0.0341933	1
24056	MAOA	2.20E-06	4.84E-05	2.0631	2.0631	Control up vs	5.10E-07	3.77516	3.77516	Control up vs	0.0602073	1.22239	1.22239	Control up vs	83.8161	6.619	0.210588	1
24139	MARCH-IV	0.0043033	0.00593026	1.93039	1.93039	Control up vs	0.00090945	2.47586	2.47586	Control up vs	0.124677	1.355	1.355	Control up vs	10.0779	2.9618	0.783706	1
24200	MBP	4.12E-05	5.20E-05	1.62308	1.62308	Control up vs	7.03E-06	1.88934	1.88934	Control up vs	0.00100777	1.36665	1.36665	Control up vs	38.7353	1.39628	0.0961243	1
24485	MGC4677	0.00546197	0.00670121	1.65541	1.65541	Control up vs	0.00088391	2.04257	2.04257	Control up vs	0.0247317	1.46699	1.46699	Control up vs	9.32121	1.68487	0.482017	1
25204	MMP9	0.00014452	0.00082983	1.86306	1.86306	Control up vs	0.00064161	1.91124	1.91124	Control up vs	0.0872078	0.791781	-1.26298	Control down	27.5175	3.70067	0.358624	1
25974	NFE2L3	0.00026331	0.00247223	1.81281	1.81281	Control up vs	8.06E-05	2.734	2.734	Control up vs	0.505353	1.10025	1.10025	Control up vs	23.2729	4.09184	0.468853	1
26113	NMU	0.00030421	0.00179608	2.04139	2.04139	Control up vs	0.00087356	2.23026	2.23026	Control up vs	0.149417	0.780078	-1.28192	Control down	22.3429	5.07506	0.605718	1
26165	NOV	1.14E-06	4.42E-07	2.70992	2.70992	Control up vs	2.78E-05	1.77912	1.77912	Control up vs	0.626534	1.03482	1.03482	Control up vs	99.2077	4.2529	0.114316	1
26923	OSAP	1.60E-06	2.00E-06	2.1757	2.1757	Control up vs	4.54E-07	2.56715	2.56715	Control up vs	0.0015629	1.35127	1.35127	Control up vs	90.9807	3.51206	0.102939	1
27734	PLAU	9.70E-05	0.0001483	1.92307	1.92307	Control up vs	4.80E-05	2.15386	2.15386	Control up vs	0.2397	1.13139	1.13139	Control up vs	30.7077	2.71663	0.235913	1
27735	PLAUR	0.00173761	0.00981175	1.99958	1.99958	Control up vs	0.00210638	2.50402	2.50402	Control up vs	0.389749	0.829381	-1.20572	Control down	13.4031	5.31325	1.05712	1
30028	SERINC2	0.00011607	0.00042313	1.68	1.68	Control up vs	1.54E-05	2.29616	2.29616	Control up vs	0.002593	1.47365	1.47365	Control up vs	29.234	2.21968	0.202474	1
30678	SLC9A2	4.18E-06	6.35E-07	2.1856	2.1856	Control up vs	6.58E-06	1.77765	1.77765	Control up vs	6.45E-05	1.52395	1.52395	Control up vs	70.9427	2.05466	0.0772326	1
30722	SLPI	0.0453135	0.0159853	2.27414	2.27414	Control up vs	0.021661	2.15537	2.15537	Control up vs	0.306857	1.34268	1.34268	Control up vs	4.24428	2.89753	1.82051	1
31228	SOX9	0.0004546	0.00417928	1.63809	1.63809	Control up vs	0.00018685	2.25006	2.25006	Control up vs	0.888794	1.01815	1.01815	Control up vs	19.9248	2.89918	0.388016	1
31369	SPRED1	1.14E-06	4.30E-06	2.19215	2.19215	Control up vs	1.76E-07	3.28596	3.28596	Control up vs	0.00024582	1.56524	1.56524	Control up vs	99.2094	4.77577	0.128369	1
31385	SPRY2	1.30E-06	7.27E-06	1.85198	1.85198	Control up vs	4.28E-07	2.44337	2.44337	Control up vs	0.0697112	1.13467	1.13467	Control up vs	95.9986	3.27663	0.0910188	1
31626	STS-1 = UBAS	1.61E-05	0.000519	1.71382	1.71382	Control up vs	2.66E-06	3.08027	3.08027	Control up vs	0.0234475	1.30917	1.30917	Control up vs	49.7485	4.3347	0.232353	1
32243	TIMP1	0.00012485	0.00017847	2.3586	2.3586	Control up vs	2.76E-05	3.05359	3.05359	Control up vs	0.0180078	1.47465	1.47465	Control up vs	28.653	4.60566	0.428637	1
32423	TMEM16A	2.71E-05	1.34E-05	1.67266	1.67266	Control up vs	1.37E-05	1.6704	1.6704	Control up vs	0.00990919	1.20197	1.20197	Control up vs	43.3628	1.21637	0.0748029	1
32623	TNC	4.34E-05	0.00043559	2.18563	2.18563	Control up vs	2.30E-05	3.29096	3.29096	Control up vs	0.957625	0.992556	-1.0075	Control down	38.1984	6.64643	0.463994	1
33015	TSPAN1	0.00018629	0.00042423	1.71618	1.71618	Control up vs	3.60E-05	2.16116	2.16116	Control up vs	0.0270584	1.28816	1.28816	Control up vs	25.6435	2.11193	0.219619	1
33461	UPK1B	2.16E-05	2.01E-05	2.12812	2.12812	Control up vs	2.97E-05	2.04602	2.04602	Control up vs	0.464053	1.0674	1.0674	Control up vs	46.0437	3.10361	0.179749	1
34673	ZNF750	1.63E-05	6.64E-06	1.68127	1.68127	Control up vs	4.84E-06	1.71907	1.71907	Control up vs	9.07E-05	1.43758	1.43758	Control up vs	49.5975	1.17418	0.0631314	1

Overlapping hits (2): DN Ras and ERKi only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

24728	MIR1974	0.0324815	0.030895	1.6235	1.6235	Control up vs	0.0365338	1.5914	1.5914	Control up vs	0.00615298	1.98049	1.98049	Control up vs	4.87925	1.56946	0.85776	1
29813	SCD	0.0136979	0.00260251	2.57632	2.57632	Control up vs	0.0943072	1.51783	1.51783	Control up vs	0.0136174	1.99862	1.99862	Control up vs	6.78813	3.07419	1.20767	1

Overlapping hits (8): GSK and ERKi only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

3184	CALB2	0.00014375	0.00058723	1.56595	1.56595	Control up vs	2.74E-05	2.01007	2.01007	Control up vs	0.00011615	1.76888	1.76888	Control up vs	27.5584	1.72869	0.167275	1
3214	CAMK2N1	0.00100495	0.0135597	1.38707	1.38707	Control up vs	0.00019664	1.95502	1.95502	Control up vs	0.00087054	1.70742	1.70742	Control up vs	15.8063	1.59537	0.269154	1
13425	KRT18P13	0.0189694	0.0449859	1.47268	1.47268	Control up vs	0.00343507	1.95168	1.95168	Control up vs	0.0166296	1.63563	1.63563	Control up vs	6.02043	1.49951	0.664187	1
13426	KRT18P17	0.0152907	0.0156297	1.59435	1.59435	Control up vs	0.00324896	1.88074	1.88074	Control up vs	0.0114795	1.64494	1.64494	Control up vs	6.52183	1.42134	0.581161	1
13869	LOC10000858	0.0411878	0.421921	1.21428	1.21428	Control up vs	0.0125685	2.08453	2.08453	Control up vs	0.0329194	1.80477	1.80477	Control up vs	4.42114	2.17872	1.31413	1
15920	LOC10013426	0.00030383	0.00162044	1.58622	1.58622	Control up vs	5.75E-05	2.14242	2.14242	Control up vs	0.00024154	1.8592	1.8592	Control up vs	22.3508	2.05063	0.24466	1
29435	RPL13	0.00181305	0.00532091	1.39585	1.39585	Control up vs	0.00063936	1.60976	1.60976	Control up vs	0.00057559	1.62237	1.62237	Control up vs	13.23	0.960086	0.193517	1
30112	SFRP1	0.0436113	0.720337	1.09813	1.09813	Control up vs	0.0209448	2.06144	2.06144	Control up vs	0.0344408	1.90085	1.90085	Control up vs	4.31472	2.574	1.59083	1

Hits (19): DN Ras only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

721	AMOT	0.0471745	0.010391	1.63628	1.63628	Control up vs	0.130396	1.28308	1.28308	Control up vs	0.505798	1.10851	1.10851	Control up vs	4.17094	0.854465	0.546298	1
1237	ASS1	0.00079265	0.00038633	1.90767	1.90767	Control up vs	0.0193644	1.38067	1.38067	Control up vs	0.68528	0.954587	-1.04757	Control down	16.9541	1.94109	0.305308	1
4096	CKMT1A	0.0017484	0.00050496	1.94487	1.94487	Control up vs	0.111011	1.2367	1.2367	Control up vs	0.801429	1.03132	1.03132	Control up vs	13.3778	1.76231	0.351291	1
4097	CKMT1B	0.00296919	0.00048698	2.14616	2.14616	Control up vs	0.156905	1.23553	1.23553	Control up vs	0.0275298	1.43925	1.43925	Control up vs	11.3486	1.94874	0.457909	1
4648	CST5	5.25E-05	3.20E-05	1.78855	1.78855	Control up vs	0.497582	1.05071	1.05071	Control up vs	0.391078	0.938832	-1.06515	Control down	36.2974	1.64767	0.12105	1
5764	EFHD1	0.00033798	0.0006408	2.02212	2.02212	Control up vs	0.00964225	1.55299	1.55299	Control up vs	0.0916512	0.779149	-1.28345	Control down	21.6855	3.44488	0.423617	1
6711	FBN2	0.00096835	0.00387026	1.60743	1.60743	Control up vs	0.105132	0.805741	-1.24109	Control down	0.0662398	0.777779	-1.28571	Control down	15.9813	2.09226	0.349119	1
6816	FDPS	0.00281079	0.00046717	1.873	1.873	Control up vs	0.00306832	1.58795	1.58795	Control up vs	0.00970645	1.45237	1.45237	Control up vs	11.5465	1.32196	0.305307	1
8778	HMGCS1	0.00180904	0.00046333	1.63339	1.63339	Control up vs	0.00088999	1.55797	1.55797	Control up vs	0.00332418	1.42773	1.42773	Control up vs	13.2389	0.924286	0.186175	1
13682	LCP1	0.00017443	9.46E-05	3.24061	3.24061	Control up vs	0.057024	1.43909	1.43909	Control up vs	0.395722	0.863274	-1.15838	Control down	26.1175	6.5663	0.670438	1
16294	LOC158160	7.55E-06	1.02E-06	1.84012	1.84012	Control up vs	5.59E-05	1.42833	1.42833	Control up vs	0.0010547	1.25932	1.25932	Control up vs	60.7865	1.2114	0.0531433	1
17532	LOC440991	7.47E-05	9.28E-06	1.6153	1.6153	Control up vs	0.00365327	1.21755	1.21755	Control up vs	0.0009474	1.27989	1.27989	Control up vs	32.9881	0.727687	0.0588241	1
20936	LOC649970	0.00066135	9.90E-05	2.2383	2.2383	Control up vs	0.0227567	1.37409	1.37409	Control up vs	0.0636233	1.27521	1.27521	Control up vs	17.8769	2.13776	0.318886	1
23934	LYPD6B	1.29E-06	2.67E-07	1.61796	1.61796	Control up vs	9.24E-07	1.50658	1.50658	Control up vs	1.54E-05	1.32613	1.32613	Control up vs	96.1462	0.843099	0.0233838	1
26287	NSDHL	7.44E-07	1.47E-07	1.61123	1.61123	Control up vs	5.74E-07	1.49227	1.49227	Control up vs	6.27E-06	1.33989	1.33989	Control up vs	110.789	0.819328	0.019721	1
30396	SLC1A3	0.00011418	4.21E-05	1.64226	1.64226	Control up vs	0.00058477	1.40255	1.40255	Control up vs	0.444383	1.05088	1.05088	Control up vs	29.3668	1.04673	0.0950484	1
31352	SPNS2	0.00036132	7.22E-05	1.74444	1.74444	Control up vs	0.00025682	1.58948	1.58948	Control up vs	0.00153119	1.4207	1.4207	Control up vs	21.2775	1.11	0.139114	1
31567	STC2	0.0014137	0.00149065	2.12659	2.12659	Control up vs	0.181103	1.2635	1.2635	Control up vs	0.163883	0.782935	-1.27725	Control down	14.2703	3.40686	0.636636	1
32301	TM7SF2	0.018213	0.00339971	1.61692	1.61692	Control up vs	0.0161305	1.42657	1.42657	Control up vs	0.0261539	1.37497	1.37497	Control up vs	6.11304	0.783381	0.341731	1

Hits (95): GSK only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

181	ABCC3	0.00055556	0.00527204	1.31193	1.31193	Control up vs	8.02E-05	1.69133	1.69133	Control up vs	0.0405273	1.19076	1.19076	Control up vs	18.8054	0.901371	0.127817	1
216	ABHD7	0.00337692	0.00216559	1.56841	1.56841	Control up vs	0.00069557	1.71775	1.71775	Control up vs	0.0121276	1.38674	1.38674	Control up vs	10.8947	1.04807	0.256534	1
227	ABLIM3	0.00014586	0.00081243	1.33868	1.33868	Control up vs	2.29E-05	1.6315	1.6315	Control up vs	0.0220482	1.17188	1.17188	Control up vs	27.4472	0.80577	0.0782855	1
622	ALCAM	0.0188281	0.595426	1.08129	1.08129	Control up vs	0.0047086	1.72931	1.72931	Control up vs	0.406978	1.13169	1.13169	Control up vs	6.03737	1.12982	0.499034	1
775	ANKRD1	0.00013439	0.00424368	1.51034	1.51034	Control up vs	7.76E-05	2.16128	2.16128	Control up vs	0.521347	0.932368	-1.07254	Control down	28.076	2.86734	0.272341	1
1004	ARAP3	0.00024799	0.00072445	1.4374	1.4374	Control up vs	4.03E-05	1.73899	1.73899	Control up vs	0.00032971	1.50564	1.50564	Control up vs	23.6691	1.03722	0.116858	1
1555	BCL2L1	5.18E-05	0.00023652	1.38993	1.38993	Control up vs	7.69E-06	1.70036	1.70036	Control up vs	0.00589839	1.21495	1.21495	Control up vs	36.4306	0.93636	0.0685401	1
1617	BHLHB2	0.00664094	0.363852	1.19558	1.19558	Control up vs	0.0058925	1.99319	1.99319	Control up vs	0.26971	0.802505	-1.2461	Control down	8.73346	2.81585	0.85979	1
1627	BID	0.00258936	0.0204315	1.26891	1.26891	Control up vs	0.00043192	1.60734	1.60734	Control up vs	0.154574	1.1387	1.1387	Control up vs	11.8477	0.757494	0.170495	1
1629	BIN1	0.00536175	0.0177921	1.38843	1.38843	Control up vs	0.00081685	1.77647	1.77647	Control up vs	0.00830695	1.46853	1.46853	Control up vs	9.3784	1.07037	0.30435	1
1634	BIRC3	0.00559912	0.89105	1.02331	1.02331	Control up vs	0.00258823	2.01758	2.01758	Control up vs	0.919852	0.983223	-1.01706	Control down	9.24505	2.2988	0.663072	1
2034	C14orf132	0.0167089	0.0609491	1.35911	1.35911	Control up vs	0.00263603	1.83065	1.83065	Control up vs	0.151962	1.24979	1.24979	Control up vs	6.31232	1.17221	0.495207	1
2116	C15orf48	0.00336606	0.529306	1.12768	1.12768	Control up vs	0.00125564	2.43068	2.43068	Control up vs	0.976513	1.00557	1.00557	Control up vs	10.9059	3.41132	0.834126	1
3517	CCL20	0.00111927	0.00487721	1.51408	1.51408	Control up vs	0.0008723	1.74206	1.74206	Control up vs	0.708786	0.959153	-1.04259	Control down	15.3072	1.66426	0.289931	1
3649	CD44	0.00095158	0.0147031	1.5304	1.5304	Control up vs	0.00012116	2.58934	2.58934	Control up vs	0.012517	1.553	1.553	Control up vs	16.0642	2.83811	0.471126	1
3672	CD82	0.00035857	0.00149215	1.4167	1.4167	Control up vs	7.22E-05	1.73268	1.73268	Control up vs	0.00025159	1.58269	1.58269	Control up vs	21.3239	1.08534	0.135727	1
3820	CEACAM1	3.67E-05	0.0005232	1.45421	1.45421	Control up vs	6.24E-05	1.66684	1.66684	Control up vs	0.107623	0.885496	-1.12931	Control down	39.9796	1.68728	0.112542	1
4109	CLCF1	0.00134307	0.00179452	1.48823	1.48823	Control up vs	0.00031254	1.68734	1.68734	Control up vs	0.0883827	1.18326	1.18326	Control up vs	14.4927	1.02149	0.187956	1
4138	CLDN7	0.0085073	0.0404217	1.37167	1.37167	Control up vs	0.00202007	1.78858	1.78858	Control up vs	0.497407	1.09634	1.09634	Control up vs	8.02841	1.25909	0.418211	1
4414	COTL1	0.00052085	0.00819763	1.55758	1.55758	Control up vs	0.00010768	2.44619	2.44619	Control up vs	0.255263	1.16835	1.16835	Control up vs	19.1594	2.89294	0.402649	1
4764	CXCL1	0.00614678	0.380768	1.22372	1.22372	Control up vs	0.00217014	2.62812	2.62812	Control up vs	0.977899	0.993798	-1.00624	Control down	8.9626	3.97715	1.18333	1
4842	CYGB	0.0506577	0.0294675	1.59482	1.59482	Control up vs	0.0117418	1.77364	1.77364	Control up vs	0.146362	1.32821	1.32821	Control up vs	4.04285	1.17887	0.777583	1

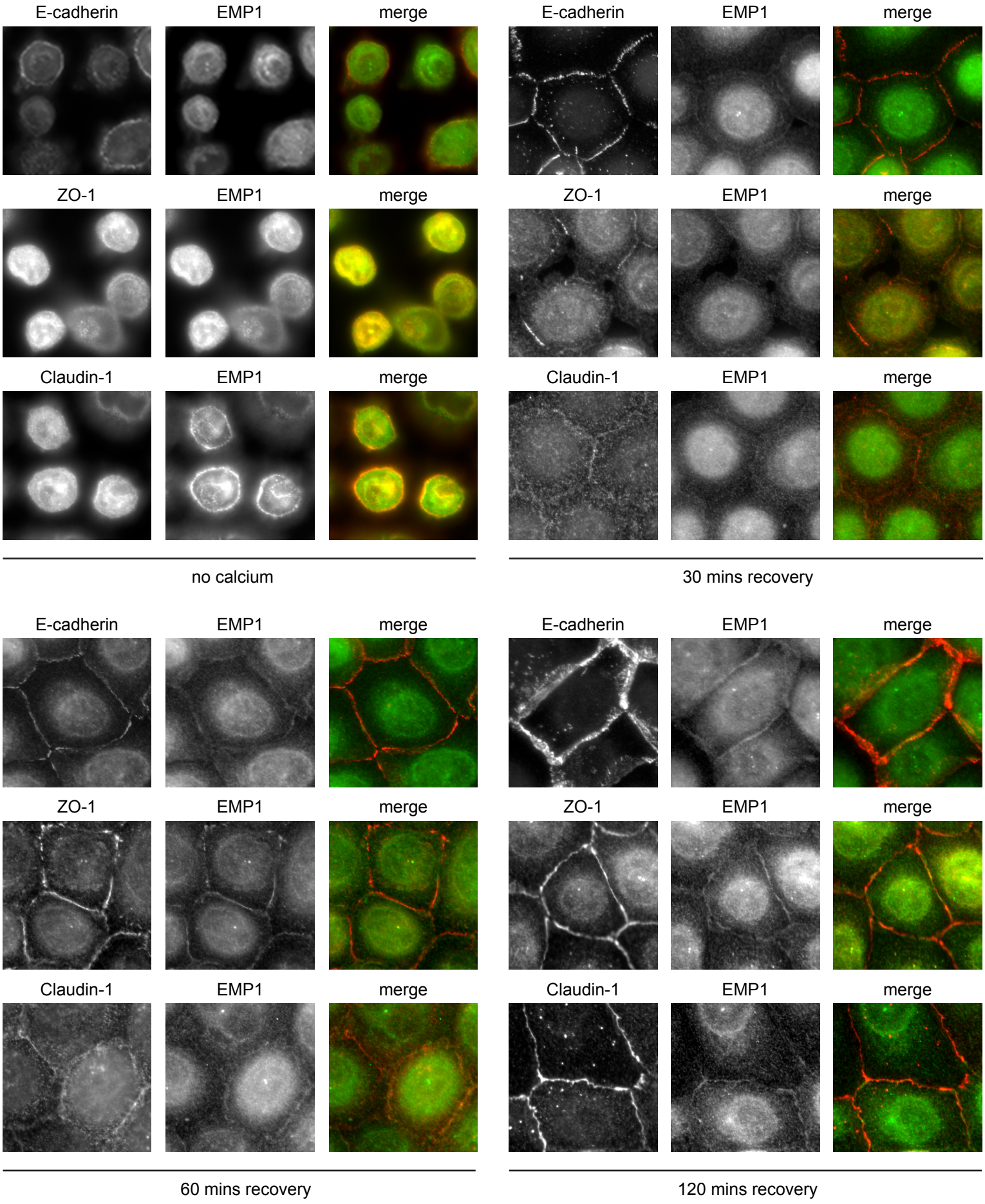
5209	DHCR24	0.0375594	0.155409	1.24937	1.24937	Control up vs	0.00629906	1.68388	1.68388	Control up vs	0.208589	1.21427	1.21427	Control up vs	4.5959	0.867314	0.503239	1			
5540	DPYSL2	0.00086808	0.0598059	1.16703	1.16703	Control up vs	0.00015714	1.60055	1.60055	Control up vs	0.176588	1.11013	1.11013	Control up vs	16.5064	0.768251	0.124114	1			
5709	ECM1	0.0311738	0.210445	1.19662	1.19662	Control up vs	0.00615862	1.6259	1.6259	Control up vs	0.407704	1.12209	1.12209	Control up vs	4.96124	0.807669	0.434122	1			
5930	EMP3	0.00527459	0.0379013	1.3524	1.3524	Control up vs	0.00144279	1.78164	1.78164	Control up vs	0.655857	1.05786	1.05786	Control up vs	9.42923	1.30485	0.369024	1			
5943	ENC1	0.00922632	0.0496746	1.59552	1.59552	Control up vs	0.00661562	2.08668	2.08668	Control up vs	0.576276	0.888872	-1.12502	Control down	7.80669	2.9905	1.02152	1			
5996	EPHA2	1.69E-07	9.08E-06	1.43133	1.43133	Control up vs	2.65E-08	2.14801	2.14801	Control up vs	0.00074921	1.21039	1.21039	Control up vs	161.844	1.98485	0.0327038	1			
6202	ETV5	6.36E-09	8.35E-09	1.58197	1.58197	Control up vs	1.10E-09	1.80829	1.80829	Control up vs	1.99E-08	1.5083	1.5083	Control up vs	371.1	1.22269	0.00878604	1			
6266	F2RL1	0.00032392	0.00082564	1.55677	1.55677	Control up vs	4.45E-05	1.97295	1.97295	Control up vs	0.00402851	1.40422	1.40422	Control up vs	21.9489	1.49127	0.181181	1			
6320	FAM107B	0.0059147	0.203091	1.18314	1.18314	Control up vs	0.0012832	1.79955	1.79955	Control up vs	0.433555	1.1052	1.1052	Control up vs	9.07829	1.25143	0.367597	1			
6373	FAM129B	5.56E-06	6.92E-06	1.56438	1.56438	Control up vs	1.05E-06	1.77486	1.77486	Control up vs	0.0003855	1.28978	1.28978	Control up vs	65.8579	1.16964	0.0473603	1			
6919	FJX1	0.00444169	0.0264724	1.29303	1.29303	Control up vs	0.00071289	1.65442	1.65442	Control up vs	0.00584245	1.42263	1.42263	Control up vs	9.97487	0.837361	0.223859	1			
7439	FOXQ1	5.75E-06	5.05E-06	1.55774	1.55774	Control up vs	1.07E-06	1.7222	1.7222	Control up vs	3.29E-05	1.41066	1.41066	Control up vs	65.2778	1.04599	0.0427299	1			
7873	GJB4	0.00015238	0.255257	1.11772	1.11772	Control up vs	0.00012561	1.86982	1.86982	Control up vs	0.157524	0.867955	-1.15213	Control down	27.1168	2.09465	0.205988	1			
8087	GPI	0.00231334	0.0164118	1.28938	1.28938	Control up vs	0.00044702	1.61602	1.61602	Control up vs	0.00182165	1.46815	1.46815	Control up vs	12.2717	0.810924	0.176215	1			
12376	IER5	0.00052863	0.0101194	1.36772	1.36772	Control up vs	7.33E-05	2.00577	2.00577	Control up vs	0.0350215	1.26754	1.26754	Control up vs	19.0775	1.56374	0.218581	1			
12453	IGFBP4	0.002303	0.206653	1.13418	1.13418	Control up vs	0.00046346	1.68319	1.68319	Control up vs	0.00912491	1.36766	1.36766	Control up vs	12.2888	0.966255	0.209677	1			
12455	IGFBP6	4.25E-06	0.38415	0.897781	-1.11386	Control down	0.00204256	1.69093	1.69093	Control up vs	1.05E-05	0.320426	-3.12084	Control down	70.6147	9.0726	0.342614	1			
12458	IGFL1	0.00018087	0.0521974	1.3983	1.3983	Control up vs	0.00140123	2.01874	2.01874	Control up vs	0.00612025	0.58093	-1.72138	Control down	25.8553	5.24315	0.540768	1			
12486	IKBKE	6.96E-05	0.00104981	1.36356	1.36356	Control up vs	1.53E-05	1.77278	1.77278	Control up vs	0.161293	1.10041	1.10041	Control up vs	33.6285	1.2104	0.0959821	1			
12555	IL27RA	3.18E-05	0.00115757	1.50476	1.50476	Control up vs	4.07E-06	2.49724	2.49724	Control up vs	0.00386961	1.39535	1.39535	Control up vs	41.5489	2.67965	0.171983	1			
12580	IL8	5.87E-05	0.0140888	1.43209	1.43209	Control up vs	9.26E-06	3.11214	3.11214	Control up vs	0.0232994	1.37892	1.37892	Control up vs	35.2132	4.35158	0.329542	1			
12739	ISG20L1	0.00074631	0.00059017	1.46461	1.46461	Control up vs	0.00014239	1.60258	1.60258	Control up vs	0.00237329	1.35618	1.35618	Control up vs	17.2565	0.784802	0.121276	1			
13309	KLF4	6.98E-05	0.0891431	1.19184	1.19184	Control up vs	0.00055105	1.65229	1.65229	Control up vs	0.00260073	0.676683	-1.4778	Control down	33.5953	2.59061	0.205632	1			
13460	KRT6A	0.00699632	0.788113	0.892028	-1.12104	Control down	0.0206236	3.26257	3.26257	Control up vs	0.0649127	0.415113	-2.40898	Control down	8.58144	13.5838	4.22114	1			
13473	KRT8	0.00518329	0.0103945	1.39239	1.39239	Control up vs	0.00075279	1.68918	1.68918	Control up vs	0.0235089	1.31987	1.31987	Control up vs	9.48361	0.878057	0.246898	1			
13675	LCN2	0.00777825	0.829099	1.09228	1.09228	Control up vs	0.00567197	4.40035	4.40035	Control up vs	0.411078	0.709502	-1.40944	Control down	8.27848	12.1431	3.91154	1			
15193	LOC100132247	0.00013875	0.0248614	1.22783	1.22783	Control up vs	1.98E-05	1.94385	1.94385	Control up vs	0.00496169	1.33098	1.33098	Control up vs	27.8296	1.44659	0.138614	1			
16246	LOC149501	0.124706	0.234823	1.2933	1.2933	Control up vs	0.0305576	1.69028	1.69028	Control up vs	0.0706909	1.51766	1.51766	Control up vs	2.59731	0.974947	1.00098	1			
19501	LOC645553	0.152919	0.777403	1.06964	1.06964	Control up vs	0.0428594	1.7396	1.7396	Control up vs	0.486754	1.18277	1.18277	Control up vs	2.31001	1.1469	1.32398	1			
23893	LTBR	0.00278643	0.0105002	1.32865	1.32865	Control up vs	0.00037161	1.65296	1.65296	Control up vs	0.0172712	1.29166	1.29166	Control up vs	11.5782	0.793229	0.182695	1			
23926	LYN	0.0353378	0.645383	1.08655	1.08655	Control up vs	0.020192	1.65176	1.65176	Control up vs	0.554344	0.898397	-1.11309	Control down	4.71362	1.33101	0.752999	1			
24032	MAL2	0.00086426	0.449206	1.08732	1.08732	Control up vs	0.00030172	1.89246	1.89246	Control up vs	0.802519	1.02758	1.02758	Control up vs	16.5279	1.71377	0.276507	1			
24091	MAP3K8	4.17E-05	0.0209179	1.14048	1.14048	Control up vs	6.25E-06	1.61226	1.61226	Control up vs	0.00277481	1.21548	1.21548	Control up vs	38.6329	0.760534	0.0524964	1			
24199	MBOAT7	0.00160045	0.540385	1.05947	1.05947	Control up vs	0.0003839	1.69589	1.69589	Control up vs	0.0897684	1.19042	1.19042	Control up vs	13.7434	1.05037	0.203805	1			
25290	MPZL2	1.44E-05	9.37E-05	1.45994	1.45994	Control up vs	1.73E-06	1.91561	1.91561	Control up vs	0.0002209	1.39699	1.39699	Control up vs	51.2639	1.33141	0.069258	1			
25631	MYO1B	0.00692329	0.264037	1.14031	1.14031	Control up vs	0.0012297	1.70411	1.70411	Control up vs	0.0898974	1.2347	1.2347	Control up vs	8.61188	0.96374	0.298422	1			
25856	NDRG1	0.00182191	0.0143807	1.32591	1.32591	Control up vs	0.00032558	1.72146	1.72146	Control up vs	0.00174841	1.5176	1.5176	Control up vs	13.2102	1.01606	0.205106	1			
25942	NET1	0.00149208	0.0111192	1.36344	1.36344	Control up vs	0.00026084	1.79461	1.79461	Control up vs	0.150328	1.16202	1.16202	Control up vs	14.0391	1.17169	0.222556	1			
25945	NEU1	0.00016787	0.00063642	1.35988	1.35988	Control up vs	2.15E-05	1.65042	1.65042	Control up vs	0.00122674	1.31925	1.31925	Control up vs	26.3977	0.797464	0.080559	1			
26477	OKL38	0.00203067	0.00243613	1.50565	1.50565	Control up vs	0.00034069	1.75016	1.75016	Control up vs	0.0186191	1.31877	1.31877	Control up vs	12.777	1.05775	0.220762	1			
27134	PARP4	0.00633359	0.0109331	1.47492	1.47492	Control up vs	0.00095028	1.8209	1.8209	Control up vs	0.0188412	1.41362	1.41362	Control up vs	8.87336	1.1557	0.347316	1			
27615	PIK3CD	0.00052854	0.00688628	1.26028	1.26028	Control up vs	7.54E-05	1.60792	1.60792	Control up vs	0.0410017	1.16876	1.16876	Control up vs	19.0784	0.733988	0.102592	1			
27628	PIM1	0.00031889	0.00112669	1.39561	1.39561	Control up vs	4.17E-05	1.72055	1.72055	Control up vs	0.00404321	1.30794	1.30794	Control up vs	22.0464	0.937931	0.113449	1			
27765	PLEK2	0.00445605	0.0944397	1.28617	1.28617	Control up vs	0.00119494	1.91564	1.91564	Control up vs	0.732936	1.048	1.048	Control up vs	9.96441	1.64306	0.439714	1			
27777	PLEKHF1	0.00061683	0.0372951	1.24225	1.24225	Control up vs	0.00010958	1.84277	1.84277	Control up vs	0.159764	1.14432	1.14432	Control up vs	18.2433	1.29275	0.188965	1			
27781	PLEKHG3	1.51E-05	2.23E-05	1.58234	1.58234	Control up vs	2.54E-06	1.84731	1.84731	Control up vs	0.0006486	1.32615	1.32615	Control up vs	50.681	1.29872	0.0683343	1			
27900	PODXL	0.00014094	0.00116186	1.55021	1.55021	Control up vs	2.92E-05	2.12414	2.12414	Control up vs	0.117027	1.16939	1.16939	Control up vs	27.7091	2.05928	0.198181	1			
28318	PROM2	0.00084246	0.0161224	1.29505	1.29505	Control up vs	0.00011216	1.81517	1.81517	Control up vs	0.00402957	1.40389	1.40389	Control up vs	16.6529	1.13001	0.180951	1			
28715	RAB38	0.00019736	0.0355184	1.18638	1.18638	Control up vs	2.90E-05	1.77344	1.77344	Control up vs	0.0101769	1.25394	1.25394	Control up vs	25.2334	1.08256	0.114405	1			
28765	RAC2	0.00054668	0.00112939	1.55992	1.55992	Control up vs	0.00010412	1.89015	1.89015	Control up vs	0.0440047	1.23955	1.23955	Control up vs	18.8933	1.43127	0.202014	1			
29127	RGS20	0.00078955	0.0012572	1.42594	1.42594	Control up vs	0.00014197	1.63958	1.63958	Control up vs	0.028307	1.21539	1.21539	Control up vs	16.9736	0.847754	0.133188	1			
29288	RNF145	2.23E-05	0.467069	1.04338	1.04338	Control up vs	6.16E-06	1.78687	1.78687	Control up vs	0.0270566	1.16205	1.16205	Control up vs	45.6629	1.32294	0.0772582	1			
29328	RNF24	0.00097642	0.0294565	1.21708	1.21708	Control up vs	0.00017605	1.62801	1.62801	Control up vs	0.200939	1.10903	1.10903	Control up vs	15.942	0.823371	0.137728	1			
29686	S100A8	0.0749563	0.427002	1.20094	1.20094	Control up vs	0.0257546	1.81824	1.81824	Control up vs	0.964623	0.990037	-1.01006	Control down	3.37618	1.51406	1.19588	1			
29687	S100A9	0.0008053	0.0950221	1.47672	1.47672	Control up vs	0.00013128	4.09738	4.09738	Control up vs	0.0523112	1.59835	1.59835	Control up vs	16.8754	6.70445	1.05944	1			
29690	S100P	0.202949	0.565142	1.20226	1.20226	Control up vs	0.0493808	2.03497	2.03497	Control up vs	0.299504	1.40582	1.40582	Control up vs	1.93228	1.70615	2.35458	1			
30034	SERPINA1	0.00034845	0.00044857	1.59022	1.59022	Control up vs	0.00011696	1.76033	1.76033	Control up vs	0.123267	1.15017	1.15017	Control up vs	21.4983	1.32837	0.164772	1			
30051	SERPINB2	0.0217282	0.0828355	1.49018	1.49018	Control up vs	0.0176389	1.82133	1.82133	Control up vs	0.533885	0.877351	-1.1398	Control down	5.71798	2.17002	1.01202	1			
30692	SLCO2A1	0.00256391	0.0458984	1.23975	1.23975	Control up vs	0.000813	1.60677	1.60677	Control up vs	0.883	1.01393	1.01393	Control up vs	11.8844	0.922421	0.206976	1			
30695	SLCO4A1	0.00135638	0.197837	1.11934	1.11934	Control up vs	0.00024112	1.6539	1.6539	Control up vs	0.0485432	1.2052	1.2052	Control up vs	14.4497	0.872189	0.160961	1			
30780	SMOX	0.00056846	0.00227331	1.34572	1.34572	Control up vs	0.00011384	1.60191	1.60191	Control up vs	0.112352	1.12776	1.12776	Control up vs	18.6808	0.795212	0.113516	1			
31370	SPRED2	7.98E-05	0.00028282	1.36998	1.36998	Control up vs	1.02E-05	1.65123	1.65123	Control up vs	0.00067602	1.31753	1.31753	Control up vs	32.392	0.802397	0.0660572	1			
31464	SSFA2	3.15E-05	7.86E-05	1.52441	1.52441	Control up vs	4.74E-06	1.85616	1.85616	Control up vs	0.00166444	1.30417	1.30417	Control up vs	41.6586	1.27772	0.08179	1			
31603	STMN3	5.98E-05	0.00013005	1.50456	1.50456	Control up vs	8.31E-06	1.81745	1.81745	Control up vs	0.00050031	1.3974	1.3974	Control up vs	35.0332	1.16452	0.0886409	1			
31766	SYT14	0.00264129	0.00731432	1.42753	1.42753	Control up vs	0.00044617	1.76864	1.76864	Control up vs	0.00282422	1.52703	1.52703	Control up vs	11.7743	1.09751	0.248566	1			
31782	SYTL4	0.00148617	0.0997896	1.15957	1.15957	Control up vs	0.0002464	1.64382	1.64382	Control up vs	0.0966944	1.16146	1.16146	Control up vs	14.056	0.833259	0.158083	1			
32134	TGFA	0.00572448	0.0162991	1.50226	1.50226	Control up vs	0.00440876	1.69325	1.69325	Control up vs	0.739327	0.954785	-1.04736	Control down	9.17744	1.55028	0.450461	1			
32139	TGFBI	0.0198036	0.255711	1.30379	1.30379	Control up vs	0.00470956	2.31554	2.31554	Control up vs	0.615474	1.11988	1.11988	Control up vs	5.92348	2.60519	1.17282	1			
32293	TM4SF1	0.00091628	0.0725257	1.26192	1.26192	Control up vs	0.00016685	2.10499	2.10499	Control up vs	0.173185	1.18325	1.18325	Control up vs	16.245	1.92664	0.316264	1			
32367	TMEM125	5.38E-05	0.00031346	1.43531	1.43531	Control up vs	1.43E-05	1.74847	1.74847	Control up vs	0.153611	1.09911	1.09911	Control up vs	36.0509	1.21357	0.0897669	1			
32407	TMEM154	0.00190736	0.0396126	1.26073	1.26073	Control up vs	0.00079902	1.63711	1.63711	Control up vs	0.81627	0.977595	-1.02292	Control down	13.0257	1.08639	0.222408	1			
32431	TMEM171	0.00010661	0.0010544	1.4786	1.4786	Control up vs	2.39E-05	1.61804	1.61804	Control up vs	0.0001183	1.46918	1.46918	Control up vs	29.9258	0.858554	0.076505	1			
32472	TMEM200A	0.016934	0.0244703	1.38816	1.38816	Control up vs	0.00295236	1.64776	1.64776	Control up vs	0.0192773	1.41393	1.41393	Control up vs	6.28109	0.827521	0.351328	1			

Hits (6): ERKi only (>1.6-fold decrease with respect to control, <0.05 unadjusted p-value)

4377	COMT	0.00538774	0.0142175	1.39561	1.39561	Control up vs	0.00595639	1.48632	1.48632	Control up vs	0.00089866	1.72934	1.72934	Control up vs	9.36344	1.0007	0.284994	1			
6691	FASN	0.00102578	0.0157442	1.35719	1.35719	Control up vs	0.00177998	1.58262	1.58262	Control up vs	0.00016006	1.94578	1.94578	Control up vs	15.7102	1.47238	0.249923	1			
15578	LOC100133323	0.00018723	0.00782308	1.25958	1.25958	Control up vs	0.00569676	1.27779	1.27779	Control up vs	2.37E-05	1.76869	1.76869	Control up vs	25.6076	1.02974	0.107233	1			
20889	LOC649821	8.12E-05	0.00077	1.29995	1.29995	Control up vs	0.00016136	1.39355	1.39355	Control up vs	1.15E-05	1.61481	1.61481	Control up vs	32.2388	0.752708	0.062261	1			
23930	LYPD3	0.00068708	0.0893153	1.1676	1.1676	Control up vs	0.00603044	1.34543	1.34543	Control up vs	0.00011281	1.75246	1.75246	Control up vs	17.6789	1.06405	0.1605	1			
25166	MLPH	0.00386949	0.010397	1.38652	1.38652	Control up vs	0.00157934	1.58331	1.58331	Control up vs	0.00106606	1.63204	1.63204	Control up vs	10.4299	0.941296	0.240666	1			

b) Possible links between Ras/MEK/ERK targets and bronchial morphogenesis

Gene	
EMP1	See main text
ERRFI1	This is a negative regulator of EGF receptor signaling and its expression is required for normal lung differentiation (Development. 2009 136:3347-56). Downregulation of ERRFI1 could lead to up regulation of EGFR signaling leading to disruption of polarity.
CRABP2	Retinoic acid (RA) and CRABP2 play a major role in normal lung development (Mol Aspects Med. 2003 24:431-40.) Loss of CRABP2 could interfere with RA signaling. Interestingly, TGM2 (tissue transglutaminase 2) and FOSL1 (transcription factor) are RA-regulated genes (Oncogene. 2003 22:4924-32.)
PHLDA1	This a stem cell marker and loss of PHLDA1 could affect the differentiation program of 16HBE cells.



Supplementary Figure 4 Recruitment during junction formation. 16HBE cells were incubated for 4 days to form mature monolayers, then subjected to a calcium switch to initiate *de novo* junction formation. During the recovery period, cells were fixed at the indicated timepoints, then co-stained for EMP1 with E-cadherin, ZO-1 or claudin-1.

Supplementary Materials and Methods:

Cloning
Dominant negative HRas N17 was subcloned into pQCXIP using Bam HI/Eco RI and fully sequenced. This mutant acts as a dominant negative with respect to all 3 mammalian Ras isoforms [30].

RNAi
An shRNA library was constructed in pSUPERpuro, comprising 3 hairpins per gene, for 86 predicted human Rho GEFs, and a Cdc42 control. EMP1 shRNAs were obtained from the TRC collection in pLKO.1 (MSKCC RNAi core facility). The specific hairpins reported in this study are as follows: shARHGEF18 (gaagctgttagtcattaca), shCdc42 (gatgacccctctactattg), shSOS1.1 (acagttgagtggcatataa), shSOS1.2 (ttacatagtagcagtctta),
shSOS1.3 (ggcagaaattcgacaatat), shEMP1.1 (ccacatcgctactgttattat), shEMP1.2 (gtgtccatctacactagtcat). The following siRNA reagents were purchased from Dharmacon: siControl (siLamin A/C; D-001620-02) and siSOS1.1 (5'-acaguugaguggcauauaauu-3')

Cell culture and treatment
16HBE cells were a kind gift from Dr Dieter Gruenert (California Pacific Medical Center, San Francisco) [28]. Cells were cultured as described previously except that FBS was purchased from Omega Scientific (FB-11, Lot 169905) [22]. BCi-NS1.1 cells were cultured in Bronchial Epithelial Growth Media (BEGM, Lonza, CA). For siRNA transfection, 2.5x10^4 16HBE cells were seeded/24 well and treated with 50nM siRNA + 1.25μl Lipofectamine LTX (Invitrogen) in antibiotic free media, for 16hrs. The medium was then replaced and the cells incubated for 4 days. For calcium-switch experiments, confluent 16HBE cells were washed 3 times in PBS and incubated in calcium free medium for 4 hrs (DMEM without $CaCl_2$, Invitrogen 21068, plus 10% FBS pretreated with Chelex 100 Resin, Biorad). Normal growth media, containing calcium, was then replaced for a further 4 hrs. Inhibitor treatments were performed using 500nM GSK1120212 (MEKi), 500nM PD0325901 or 1μM Sch772984 (ERKi); DMSO (1:20,000) was used as a carrier control. GSK1120212 and PD0325901 were purchased from Selleck; SCH772984 was kindly provided by Neal Rosen.

Virus production and preparation of stable cells
Retroviral or lentiviral particles were prepared to deliver cDNA or shRNA for stable expression, as described previously [52]. 16HBE infections were performed by centrifugation. 10^5 cells were seeded in a 6-well dish, incubated with 1.5ml of viral suspension + 8ug/ml polybrene and spun at 2250rpm for 30mins. Two days post-infection, stable pools were selected using 1.5μg/ml puromycin for 4-5 days. To avoid clonal effects, experiments were performed with freshly prepared, short term cultures of selected stables pools, and separate pools were used for each independent experimental repeat. Stable cells were typically reseeded on glass coverslips or plastic dishes for subsequent experiments. However, expression of HRas N17 prevented reattachment after trypsinisation, so these cells were reseeded on coverslips/plastic 1 day post-infection, and selected directly. For BCi-NS1.1, cells were infected overnight in media + 2ug/ml polybrene with purified, high titer lentivirus, harbouring control or EMP1 specific shRNA, at an equal multiplicity of infection.

QPCR
10^5 cells were seeded per 6-well. The media was replaced after 24hrs, -/+ inhibitors, and the cells were incubated for a further 3 days. RNA was prepared using the RNeasy kit, with an on-column DNase I digestion, according to the manufacturer's instructions (Qiagen). cDNA was synthesized using Superscript III First-Strand Synthesis Supermix, with random hexamers (Invitrogen, 18080-400). QPCR was performed using Taqman Universal PCR mastermix (Applied Biosystems, 4304437) and the following probes (Applied Biosystems): EMP1 (Hs00608055_m1) and GAPDH (Hs02758991). Samples were loaded onto a 96-well plate (Applied Biosystems, 4346906) and analyzed using a Biorad iQ5 Multicolor RT-PCR Detection System. The data represent 3 independent experiments, each performed in triplicate, with EMP1 expression normalized against the GAPDH control.

Western blotting
Total cell extracts were prepared and western blotting was performed as described previously [53]. Primary antibodies were used as follows: mouse anti-beta actin (AC-74, Sigma), 1:50,000; mouse anti-pERK (M8159, Sigma), 1:1000; rabbit anti-total ERK (M5670), 1:2000; rat anti-Ras (sc-35, Santa Cruz), 1:1000; rabbit anti-p90RSK (S380, Cell Signaling, 9335P); rabbit anti-RSK1 (D6D5, Cell Signaling, 8408S); rabbit anti-SOS1 (sc-256, Santa Cruz), 1:1000. HRP-conjugated secondary antibodies (Dako) were used at 1:5000. Blots were scanned using a Biorad GS-800 Calibrated Densitometer and molecular weight markers are indicated in figures. All western blots are representative of data obtained from three independent experiments.

Immunofluorescence & epifluorescent imaging
16HBE cells were grown on glass coverslips and incubated as described in figure legends. All subsequent steps were performed at room temperature in PBS, unless otherwise indicated. For ZO-1, occludin and E-cadherin staining, cells were fixed using 3.7% formaldehyde (10mins). For MEK, pERK or EMP1 staining, cells were fixed with MeOH at -20°C (10mins). Cells were permeabilized using 0.5% Triton X-100 (5mins), rinsed and then stained with primary antibody overnight at 4°C. Primary antibodies were used as follows: rat anti-E-cadherin (13-1900, Invitrogen), 1:100; mouse anti-EMP1 (Abnova, H00002012-A01), 1:100; rabbit anti-EMP1 (Abgent, AP17400a), 1:100; rabbit anti-pERK (4370, Cell Signaling); mouse anti-MEK (4694, Cell Signaling), 1:100; rabbit anti-ZO-1 (61-7300, Invitrogen), 1:25-100 (according to lot#). Cells were washed, incubated with Alexa Fluor 488/568 goat anti-rabbit (H+L) secondary (1:500; Invitrogen) and Hoechst 33342 (1μg/ml) for 45mins, washed again with PBS and then with water. Coverslips were mounted using Aqua Polymount (Polysciences, Inc) and visualized using an upright AX10 Imager.A1 epifluorescent microscope (Zeiss), equipped with an EC-Plan-NEOFLUAR 20x/0,5 objective (420350-9900) or a Plan-APOCHROMAT 63x/1,4 oil objective (420780-9900) and a Hamamatsu Orca-ER 1394 C4742-80 camera, controlled by Axiovision software (Zeiss). Scale bars were added using Metamorph software (Molecular Devices). All images are representative of data obtained from three independent experiments.

16HBE tight junction formation assay
16HBE cells were seeded at 2.5x10^4 cells/coverslip in a 24-well plate, treated -/+ inhibitors, and incubated for 3-4 days. Cells were fixed and stained for ZO-1/DNA.

For each sample, 5 random, non-overlapping images were acquired at 20x as described above; >500 cells were counted/condition/experiment, across three independent experiments. Apical junction formation was quantified using Metamorph software. Cells with a continuous ring of ZO-1 at cell-cell contacts were counted as having intact apical junctions, cells with punctate, discontinuous or absent ZO-1 at cell-cell contacts were defined as not having apical junctions.

BCi-NS1.1 tight junction formation assay

BCi-NS1.1 cells were seeded at 4.5 x10^5 cells/cm^2 on transwell inserts (0.4 μm size pore; Corning) coated with human type IV collagen (Sigma) in media consisting of a 1:1 mixture of DMEM (Cellgro, Manassas, VA) and Ham's F-12 Nutrient Mix (Invitrogen) supplemented with 100 U/ml penicillin, 5% fetal bovine serum 100 μg/ml streptomycin, 0.1% gentamycin and 0.5% amphotericin B. Following overnight incubation, the media was replaced with 1:1 DMEM/Ham's F12 (including antibiotics described above) supplemented with 2% of the serum substitute Ultroser G (BioSerpa S.A., Cergy-Saint-Christophe, France). Two days post seeding, the cells were fixed and stained for ZO-1/DNA. For each sample, z-stacks were acquired from 6 non-overlapping fields of view; this was repeated across 3 independent experiments. Images were acquired using a Nikon Ti-E inverted microscope attached to a CoolSNAP CCD camera (Photometrics) with a ELWD 20x Plan Fluor objective and NIS Elements software (Nikon). Nuclei were counted to confirm that cell number was consistent across different stable cell lines. Apical junction formation was quantified by counting continuous rings of ZO-1 staining/field of view.

Transepithelial resistance assay

5x10^4 16HBE cells were seeded on collagen coated, 0.4μm PTFE membrane filters in 6.5mm inserts (Costar, 3495), on 24-well plates (Corning). 24 hours later, the cells were confluent and the media was replaced -/+ inhibitors for a further 3 days. Transepithelial resistance was measured using an EVOM voltohmmeter and STX2 electrode (World Precision Instruments), according to the manufacturer's instructions. The data were acquired through three independent experiments. For BCi-NS1.1 cells, air-liquid interface (ALI) cultures were analyzed on day 21 post-seeding, as described previously [42].

FM4-64 dye assay and confocal imaging

10^5 16HBE cells were seeded per glass bottomed, 35mm dish (MatTek). 24 hours later, the media was replaced -/+ inhibitors, and the cells were incubated for a further 3 days. The lipophilic probe, FM4-64 (5μg/ml; Invitrogen) was added to the media for 10mins at 37°C. Confocal z-stacks were acquired from 10 fields of view using an Ultraview Vox spinning disc confocal system (Perkin Elmer), equipped with a Yokogawa CSU-X1 spinning disc head, and EMCCD camera (Hamamatsu C9100-13), coupled with a Nikon Ti-E microscope. For quantification, 3 x/z slices per image were analyzed using ImageJ software. To generate fluorescence ratios, line profiles were drawn over apical and basolateral surfaces to calculate average fluorescent intensities. The data were acquired through three independent experiments.

Microarray analysis

Differential gene expression analysis was performed using 4 parallel samples: control (pQCXIP cells, DMSO), dominant negative HRas (pQCXIP-HRas N17 cells), MEK inhibitor (pQCXIP cells, GSK1120212/MEKi), ERK inhibitor (pQCXIP cells,

SCH772984/ERKi). The cells were seeded at 10^5/6-well. 24 hours later, the medium was replaced -/+ inhibitors, and the cells were incubated for a further 3 days. Three independent samples were prepared for each condition. RNA was isolated as described above. Quality control and microarray analysis were performed by the MSKCC Genomics Core Laboratory, using an Illumina gene expression array (Human HT-12, 47 000 transcripts). The data were analyzed using Partek software. Briefly, gene level data were subjected to quantile normalization and each group was compared to the control to detect differentially expressed genes using 1-way ANOVA. We selected genes that were downregulated by >1.6-fold, relative to the control, with an unadjusted p-value <0.05.

Statistics

Unless otherwise indicated, statistical significance was evaluated using Prism software.

Unpaired t-tests were performed, with 2-tailed P-values and 95% confidence intervals.