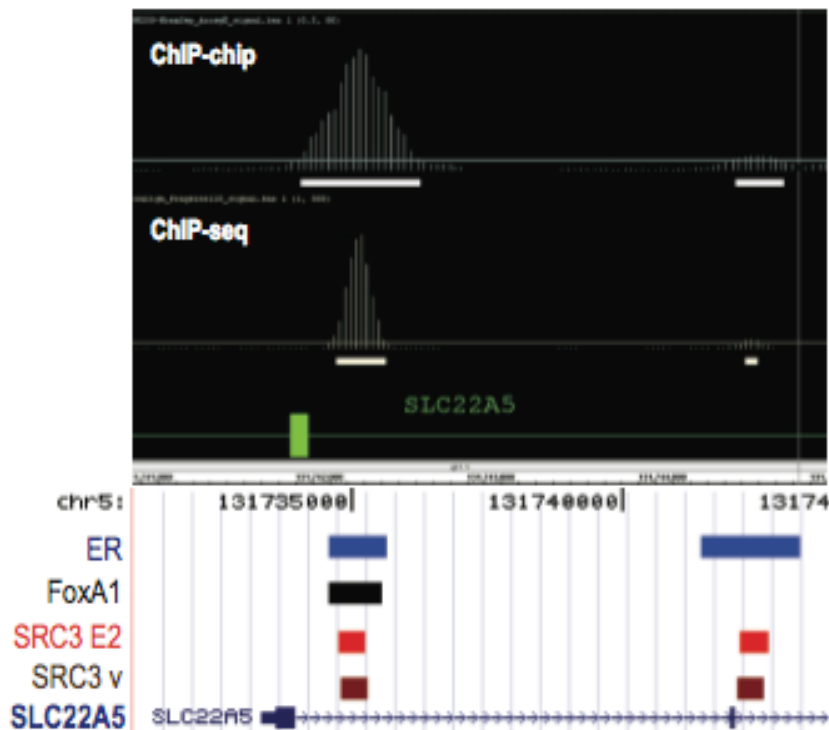


Supplementary Material 3: Validation Experiments

A) Identification of SRC-3 and RNA Polymerase II Binding Sites in MCF-7 Breast Cancer Chromatin by ChIP-chip

We previously used Genpathway's FactorPath and TranscriptionPath methods to identify possible SRC-3 target genes in MCF-7 cells [1]. To better understand the differences between the ChIP-seq and ChIP-chip approaches in this work, we replicated the genomic mapping for SRC-3 high affinity sites by hybridizing LM-PCR amplified SRC-3 DNA from E2 treated MCF-7 cells to the Affymetrix tiled microarrays. Data analysis using a threshold that produced a FDR of 1.29% identified a total of 6,364 SRC3_E2 sites and 2,847 potential target genes that had SRC-3 tag sequences within a 10Kb gene margin. 2,538 tag sequences (about 40%) were not within 10Kb of any annotated genes (data not shown). Comparative analyses using different threshold settings showed that the different readouts in the two ChIP platforms produced highly compatible results, indicating that the bias introduced by amplification of the DNA fragments in the ChIP-chip method was negligible in this case. The figure below illustrates an example of a good peak correlation between the two ChIP platforms for the SLC22A5 gene, and the perfect alignment in the UCSC Genome Browser [2] of the SRC-3 tag sequences with ER and FoxA1 binding sites from the re-calculated ER and FoxA1 cistromes. For the analysis of the SRC-3 cartography presented in the paper we simply used the SRC3-ChIP-seq data.



B) IP Validation by Q-PCR

Real-time quantitative PCR on 126 randomly selected SRC-3 and Pol II ChIP target DNAs validate our ChIP assays; we found only one gene (RAPGEF1) for which recruitment was not increased upon E2 treatment of the MCF-7 cells. The SLC22A5 gene shown in the above figure, for example, measured a 42.5 fold increase in DNA over vehicle control sample.

Name	hg18	start	end	Gene	QPCR NORM to Untreated	NORM Std Dev
Val-1	chr1	17719363	17719889	FLJ10521	16.51	2.79
Val-2	chr1	21440761	21440962	ECE1	19.28	0.92
Val-3	chr1	22671608	22671962	KIAA0478	27.01	2.42
Val-4	chr1	42098221	42098678	HIVEP3	21.66	4.72
Val-5	chr1	43176130	43176416	SLC2A1	1.92	2.19
Val-6	chr1	44069224	44069353	SIAT6	32.47	6.51
Val-7	chr1	108111313	108111862	VAV3	5.82	1.74
Val-8	chr1	108543172	108544297	SLC25A24	7.91	0.09
Val-9	chr1	114925317	114925887	BCAS2	1.72	0.27
Val-10	chr1	120375851	120376801	NOTCH2	9.46	0.89
Val-11	chr1	144434835	144435296	CD160	13.98	0.97
Val-12	chr1	162983974	162984653	PBX1	6.97	1.59
Val-13	chr1	167765202	167765567	F5	31.74	8.10
Val-14	chr1	177252240	177253052	FAM20B	2.39	0.08
Val-15	chr1	200505460	200505697	LGR6	2.04	0.07
Val-16	chr1	205143512	205143895	IL24	17.88	3.45
Val-17	chr1	216699867	216700095	TGFB2	2.83	0.49
Val-18	chr10	27189497	27190429	ABI1	1.33	0.64
Val-19	chr10	43988744	43989355	Clust10-9	20.00	*
Val-20	chr10	51217247	51217864	MSMB	11.31	0.63
Val-21	chr10	92670915	92671209	ANKRD1	4.75	0.88
Val-22	chr10	95184871	95185372	FER1L3	20.00	*
Val-23	chr10	104460260	104460798	ARL3	55.40	9.42
Val-24	chr10	104918097	104918341	NT5C2	15.33	0.36
Val-25	chr11	20009901	20010303	NAV2	19.75	2.18
Val-26	chr11	30499374	30500156	C11orf8	45.41	4.40
Val-27	chr11	77648626	77649176	GAB2	41.89	4.65
Val-28	chr11	94218282	94218837	AMOTL1	12.72	0.85
Val-29	chr12	1746892	1747759	ADIPOR2	15.02	2.92
Val-30	chr12	51625148	51626471	KRT18	10.92	0.90
Val-31	chr12	52484267	52484890	LOC440100	12.03	0.91
Val-32	chr13	38156703	38157327	FREM2	20.00	*
Val-33	chr14	73845357	73845757	ABCD4	6.25	1.38
Val-34	chr15	37747821	37748288	FSIP1	20.00	*
Val-35	chr15	62025103	62025468	DAPK2	9.27	1.34
Val-36	chr15	70215796	70215961	SENP8	1.31	0.21
Val-37	chr15	72721983	72722086	FLJ21128	6.33	0.90
Val-38	chr15	87465608	87465974	ABHD2	13.16	2.05
Val-39	chr15	94681200	94681490	NR2F2	5.23	0.84

Val-40	chr16	73901356	73901558	CFDP1	5.82	1.18
Val-41	chr17	1891864	1892108	OVCA2	1.00	0.83
Val-42	chr17	9813008	9813203	GAS7	5.46	0.04
Val-43	chr17	24429824	24429996	MYO18A	3.98	0.74
Val-44	chr17	35858346	35858767	IGFBP4	14.06	0.98
Val-45	chr17	36906475	36907031	KRTHA6	2.85	0.56
Val-46	chr19	2475911	2476765	GNG7	6.29	0.16
Val-47	chr19	7393000	7393142	ARHGEF18	5.36	1.17
Val-48	chr19	9818468	9818572	PIN1	3.54	0.64
Val-49	chr19	14816134	14816233	OR7A10	4.25	0.96
Val-50	chr19	15457560	15458276	PGLYRP2	8.15	1.53
Val-51	chr19	16794925	16795402	SIN3B	7.03	1.83
Val-52	chr19	17763255	17763778	B3GNT3	4.56	0.62
Val-53	chr19	40943635	40943839	HSPB6	2.63	1.54
Val-54	chr19	44020435	44020576	HNRPL	8.05	0.86
Val-55	chr19	45029821	45030018	FBL	2.24	1.31
Val-56	chr19	49937019	49937840	BCL3	7.23	0.68
Val-57	chr19	52120630	52120756	GRLF1	5.30	1.51
Val-58	chr19	52347470	52347627	SAE1	9.11	1.06
Val-59	chr19	52531710	52532508	GPR77	12.39	0.32
Val-60	chr2	11589775	11591494	GREB1	23.55	4.99
Val-61	chr2	43532909	43533587	THADA(1)	38.27	4.21
Val-62	chr2	43558990	43559767	THADA(2)	14.64	4.51
Val-63	chr2	238075929	238076157	MLPH	15.35	0.96
Val-64	chr2	240658125	240658298	Clust2_36	20.00	*
Val-65	chr20	30092111	30092531	HCK	14.14	3.17
Val-66	chr20	42776664	42777175	WISP2	6.76	1.77
Val-67	chr20	48777155	48777963	PARD6B	20.00	*
Val-68	chr20	48813577	48814669	PARD6B	10.00	*
Val-69	chr20	51650499	51651859	ZNF217	2.89	0.22
Val-70	chr20	51916416	51916594	slervar	10.00	*
Val-71	chr20	57762529	57762683	PHACTR3	1.38	0.10
Val-72	chr21	32789694	32789842	C21orf63	13.56	2.67
Val-73	chr21	35022864	35023000	CLIC6	8.29	1.84
Val-74	chr21	38637655	38637800	ERG	5.54	0.59
Val-75	chr21	40348272	40348505	DSCAM-K	2.78	0.27
Val-76	chr21	40364567	40365125	DSCAM-J	3.16	1.29
Val-77	chr21	40368398	40368940	DSCAM-I	5.86	1.72
Val-78	chr21	40608133	40608590	DSCAM-H	2.69	0.71
Val-79	chr21	40614441	40615128	DSCAM-G	6.21	0.74
Val-80	chr21	40618192	40619029	DSCAM-F	28.56	2.40
Val-81	chr21	40633827	40634940	DSCAM-E	13.11	2.14
Val-82	chr21	40661772	40661908	DSCAM-D	1.44	0.99
Val-83	chr21	40673465	40674396	DSCAM	5.24	0.87
Val-84	chr21	40676456	40676865	DSCAM-C	7.81	0.21
Val-85	chr21	40680513	40681154	DSCAM-B	2.23	0.07
Val-86	chr21	40737823	40737970	DSCAM-A	1.33	0.13
Val-87	chr21	41616235	41616379	FAM3B	5.59	1.17
Val-88	chr21	42656549	42656988	TFF1 (ln1)	5.00	*

Val-89	chr21	42659864	42660070	TFF1	20.00	*
Val-90	chr21	42668505	42669854	TMPRSS3	27.68	2.19
Val-91	chr21	43956437	43957118	PDXK	9.01	1.60
Val-92	chr21	45350388	45350580	ADARB1	3.47	0.77
Val-93	chr21	45574636	45574745	C21orf93	12.61	2.84
Val-94	chr22	28207795	28208167	NEFH	5.23	0.64
Val-95	chr22	37650351	37650478	APOBEC3A	4.99	0.60
Val-96	chr3	54362640	54363203	CACNA2D3	20.00	
Val-97	chr3	61718231	61718783	PTPRG	2.79	0.49
Val-98	chr3	64451785	64452597	ADAMTS9	4.16	0.12
Val-99	chr3	126264200	126265012	SLC12A8	15.27	0.46
Val-100	chr3	158013023	158013164	LOC389169	22.81	0.14
Val-101	chr3	158013228	158013361	FLJ16641	7.21	0.33
Val-102	chr3	158367042	158367264	CCNL1	2.59	0.09
Val-103	chr3	195359472	195359789	HES1	15.49	5.04
Val-104	chr4	15273512	15273710	FBXL5	2.57	0.82
Val-105	chr4	38721444	38721576	KLHL5	10.42	0.65
Val-106	chr4	90058656	90058787	FAM13A1	1.07	0.25
Val-107	chr4	140806643	140806808	MGST2	2.77	0.64
Val-108	chr4	140989921	140990207	MAML3	8.49	1.27
Val-109	chr4	141390941	141391054	SCOC_prom	6.47	0.40
Val-110	chr4	141446117	141446265	SCOC_In1	1.46	0.20
Val-111	chr5	35224055	35224270	PRLR	15.12	4.03
Val-112	chr5	131742208	131742620	SLC22A5	42.47	1.00
Val-113	chr5	132612580	132613575	FSTL4	6.39	0.89
Val-114	chr6	17497510	17497918	CAP2	20.00	*
Val-115	chr6	30827236	30827658	IER3	20.00	*
Val-116	chr6	53437352	53438686	Clust6-7	20.00	*
Val-117	chr7	116551861	116552217	ST7	20.49	0.89
Val-118	chr7	139116517	139119489	LOC389562	10.00	*
Val-119	chr8	11625546	11625974	GATA4	27.76	5.29
Val-120	chr8	79605057	79605509	PKIA	13.14	5.78
Val-121	chr8	122232288	122232720	bobly	20.00	
Val-122	chr8	145102690	145102874	PLEC1	8.37	0.37
Val-123	chr9	33405706	33405930	AQP7	14.40	2.97
Val-124	chr9	84846624	84846954	RASEF	10.56	0.57
Val-125	chr9	100349899	100350088	GPR51	12.53	3.74
Val-126	chr9	133578150	133578483	RAPGEF1	0.98	0.07

* strong binding (query with different normalization)

1. Labhart, P., et al., *Identification of target genes in breast cancer cells directly regulated by the SRC-3/AIB1 coactivator*. Proc Natl Acad Sci U S A, 2005. **102**(5): p. 1339-44.
2. Kent, W.J., et al., *The human genome browser at UCSC*. Genome Res, 2002. **12**(6): p. 996-1006.

