Supporting Information

Nurr1 modulation mediates neuroprotective effects of statins

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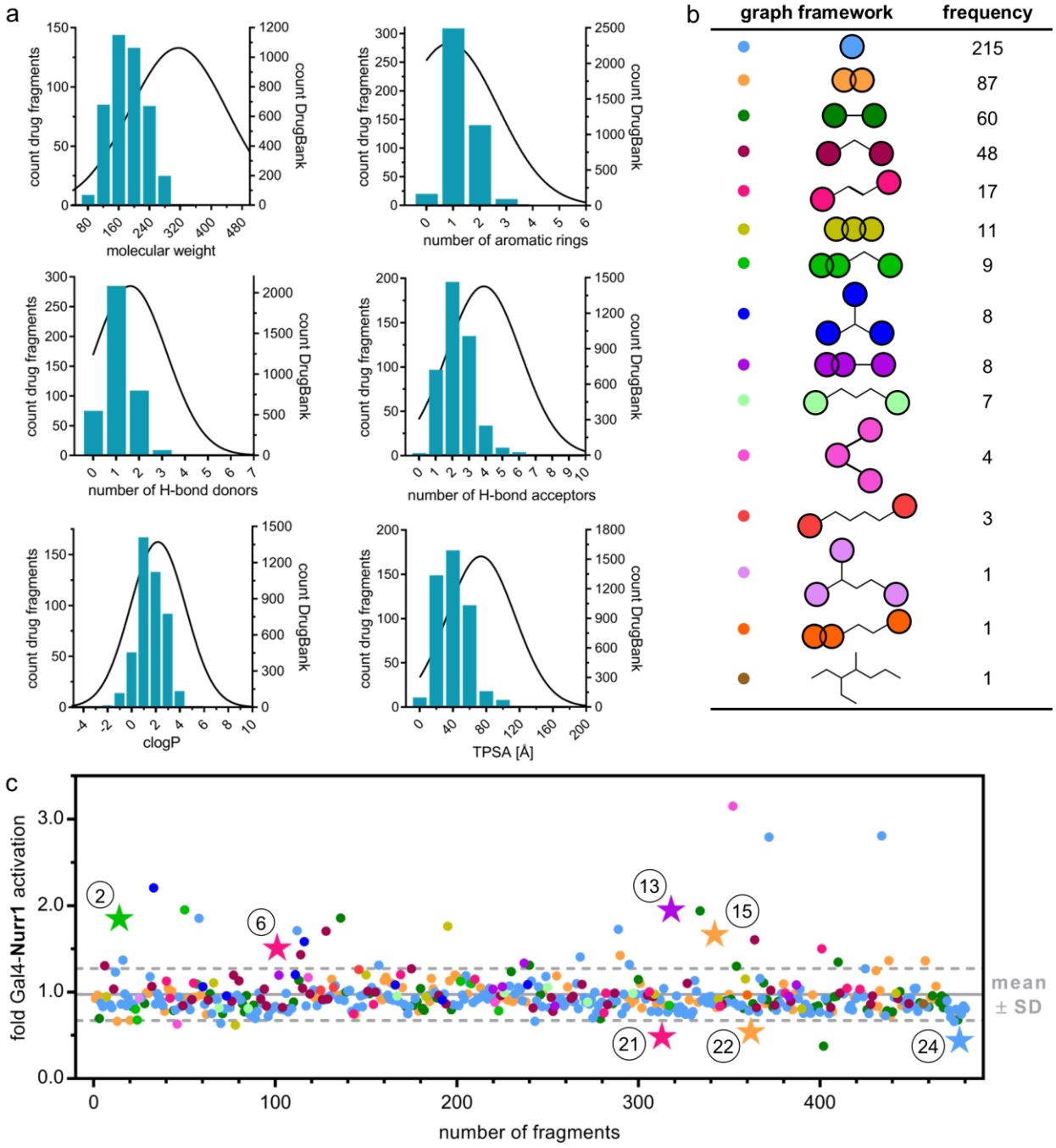
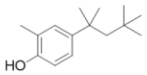
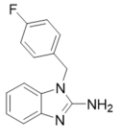
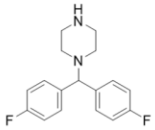
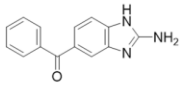
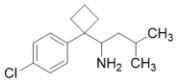
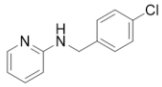
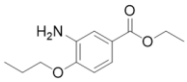
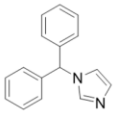
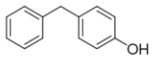
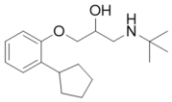
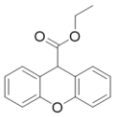
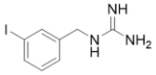
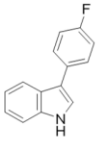
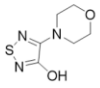
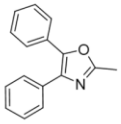
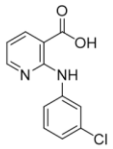
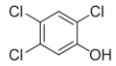
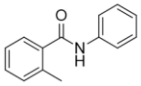
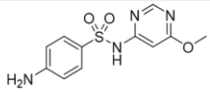
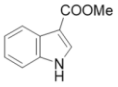
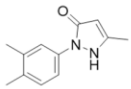
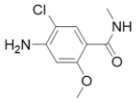


Figure S1. (A) Feature distributions of the drug fragment library (N=480) in comparison with the DrugBank database (N=7946). Bars are plotted on the left y-axis for Prestwick drug fragments. Gaussian distributions of DrugBank compounds (MW ≤ 500) are plotted on the right y-axis. (B) Representations of the different graph frameworks contained in the drug fragment library and their frequencies. (C) Nurr1 modulatory activity of the drug fragment library in a Gal4 hybrid Nurr1 reporter gene assay. Results from primary screen are the mean reporter activity vs. 0.4% DMSO; n=2. Fragments affecting reporter activity ≥1.5-fold (Nurr1 activation, **1**-**20**) or ≤0.6-fold (Nurr1

repression, **21**-**24**) were considered for further evaluation as primary screening hits. Labeled compounds marked with a star relate to the fragment hits validated in control experiments on Gal4-VP16. Different colors refer to different graph frameworks (from B). Gray lines represent mean±SD of the entire screening.

a

fragment hit	structure	primary screen (reporter act. at 100 μM)	Gal4-Nurr1 modulation
● 1		4.16±5.23	toxic in primary screen
★ 2		1.85±0.04	EC_{50} 11±6 (1.98±0.21 max. act.)
● 3		2.21±0.83	inactive (50μM) toxic (≥ 100 μM)
● 4		1.95±0.33	inactive (50 μM) toxic (≥ 100 μM)
● 5		1.85±0.31	inactive (30 μM) toxic (≥ 50 μM)
★ 6		1.51±0.36	EC_{50} 39±14 (1.82±0.14 max. act.)
● 7		1.71±0.07	inactive (50 μM) toxic (≥ 100 μM)
● 8		1.59±0.29	inactive (30 μM) toxic (≥ 50 μM)
● 9		1.71±0.02	toxic in primary screen
● 10		1.86±0.43	inactive (30 μM) toxic (≥ 100 μM)
● 11		1.76±0.10	inactive(50 μM) toxic (≥ 100 μM)
● 12		1.73±0.35	toxic in primary screen

fragment hit	structure	primary screen (reporter act. at 100 μM)	Gal4-Nurr1 modulation
★ 13		1.95±1.44	EC_{50} 7.7±3.2 (2.48±0.18 max. act.)
● 14		1.94±0.24	inactive (10 μM) toxic (≥ 30 μM)
★ 15		1.66±0.33	EC_{50} 25±10 (1.89±0.14 max. act.)
● 16		3.15±0.79	inactive (10 μM) toxic (≥ 30 μM)
● 17		1.60±0.23	inactive(10 μM) toxic (≥ 30 μM)
● 18		2.79±0.00	toxic in primary screen
● 19		1.50±0.05	inactive (100 μM)
● 20		2.81±0.32	toxic in primary screen
★ 21		0.48±0.05	IC_{50} 71±32 (0.25±0.12 rem. act.)
★ 22		0.54±0.06	IC_{50} 48±11 (0.31±0.08 rem. act.)
● 23		0.38±0.02	inactive (30 μM) toxic (≥ 50 μM)
★ 24		0.44±0.03	IC_{50} 54±11 (0.18±0.06 rem. act.)

b

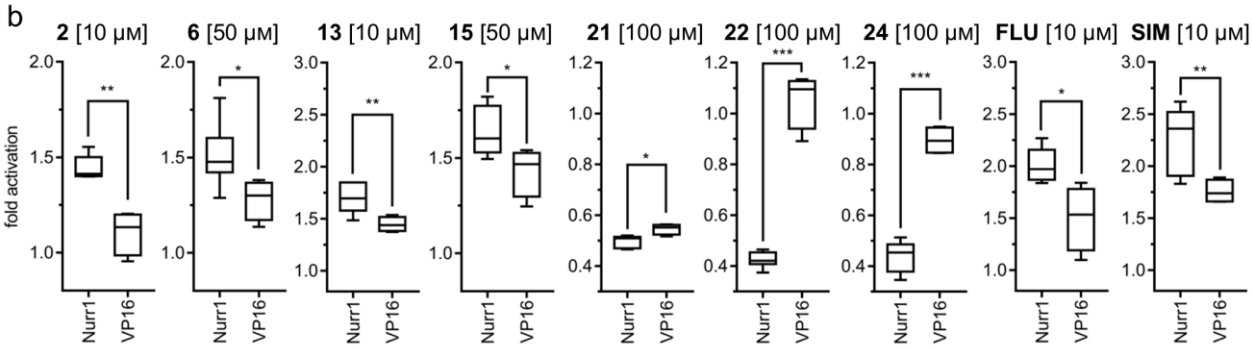
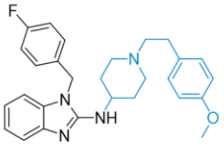
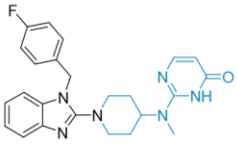
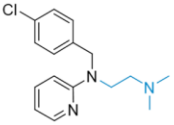
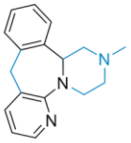
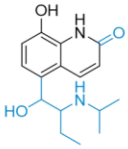
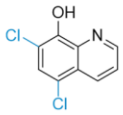


Figure S2. Follow up of the primary drug fragment screen for Nurr1 modulation. (A) 24 primary screening hits further considered and their Nurr1 modulatory activity in a Gal4-Nurr1 hybrid reporter gene assay. Reporter activity in primary screen is mean±SD reporter activity, n=2. Nurr1 modulation: only activities validated against Gal4-VP16 are reported. Data for fragment **22** from [1].EC_{50} or IC_{50} values are mean±S.E.M.; n≥3. Maximum activation or remaining activity refers to the maximum reporter activation or repression efficacy compared to DMSO (0.1%) treated cells. Toxic false positive hits from the initial screen were not further investigated. (B) Control experiments employing a Gal4-VP16 hybrid receptor were performed to confirm or refute Gal4-Nurr1 mediated activity in the cellular hybrid reporter gene assay. Boxplots show: center line, median; box limits, upper and lower quartiles; whiskers, min/max; $n \geq 4$. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ (t-test).

a

drug INN (related fragement)	structure	Nurr1 modulation
Astemizole (from **2**)		inactive (10 µM) toxic (≥ 30 µM)
Mizolastine (from **2**)		inactive (30 µM) toxic (≥ 100 µM)
Chlorpyramine (from **6**)		inactive (100 µM)
Mirtazapine (from **6**)		inactive (100 µM)
Procaterol (from **15**)		inactive (100 µM)
Chloroxine (from **15**)		toxic (≥ 10 µM)

b

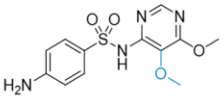
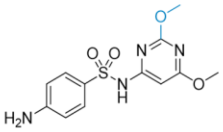
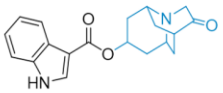
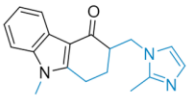
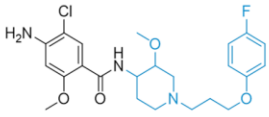
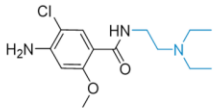
drug INN (related fragement)	structure	Nurr1 modulation
Sulfadoxine (from **21**)		IC_{50} 77±26 µM (0.43±0.09 rem. act.)
Sulfadimethoxine (from **21**)		IC_{50} 15±4 µM (0.37±0.05 rem. act.)
Dolasetron (from **22**)		inactive (100 µM)
Ondansetron (from **22**)		inactive (100 µM)
Cisapride (from **24**)		inactive (100 µM)
Metoclopramide (from **24**)		inactive (100 µM)

Figure S3. Nurr1 modulatory activity of fragment derived drugs. (a,b) FDA approved drugs related to validated Nurr1 activating (a) and repressing (b) fragments were tested on Nurr1 in a Gal4-Nurr1 hybrid reporter gene assay. Structural extensions compared to the underlying fragments are shown in blue. Only statins and the antibiotics sulfadoxine and sulfadimethoxine retained the Nurr1 modulatory activity of their fragment precursors **13** and **21** (see also Figure 1b). IC_{50} values are mean±S.E.M.; n≥3.

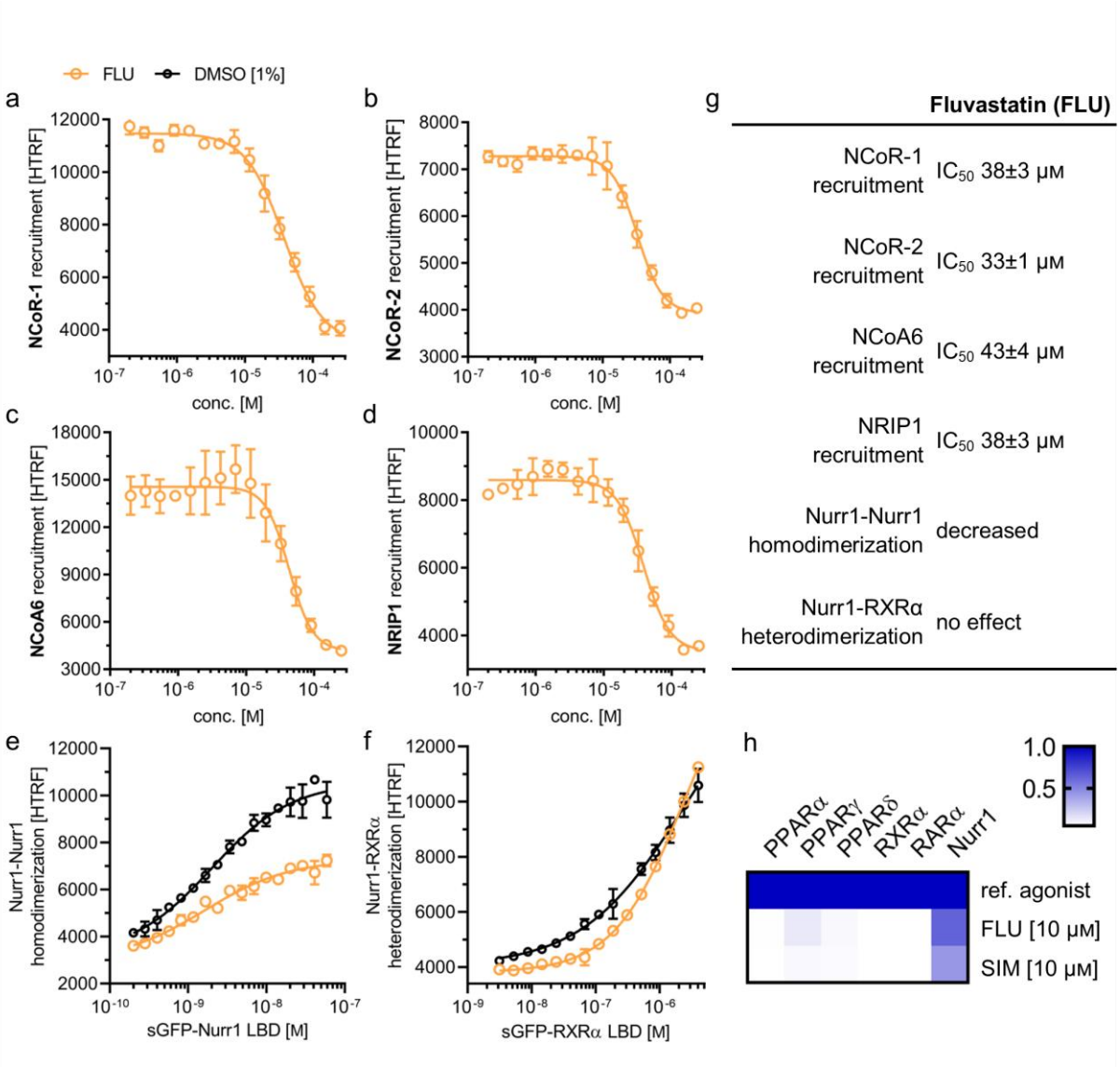


Figure S4. Effects of fluvastatin (FLU) on co-regulator interactions and dimerization of Nurr1. (a-d) Fluvastatin displaced NCoR-1 (a), NCoR-2 (b), NCoA6 (c) and NRIP1 (d) from the Nurr1 LBD. (e, f) Fluvastatin (30 µM) decreased homodimerization of Nurr1 (e) without affecting Nurr1-RXRα heterodimerization (f). Data are the mean±SD; N=3. (g) Summarized cell-free Nurr1 modulatory activities of FLU. (h) Selectivity profiles of FLU and SIM at 10 µM on related lipid-activated transcription factors in Gal4 hybrid reporter gene assays. Heatmap shows mean relative activation compared to reference agonists at 1 µM for PPARs (α: GW7647; γ: rosiglitazone; δ: L165,041), RXRα (bexarotene), RARα (tretinoin) and 100 µM for Nurr1 (amodiaquine); n ≥ 2.

Table S1. Effects of Simvastatin on differential gene expression in T98G cells. Only protein coding genes with log_2fold change > |0.5| that were selectively altered by Simvastatin in nt siRNA treated cells but not altered in Nurr1 silenced cells are shown.

Gene name	Gene description	log_2 (fold change)	$-log_{10}$ (p value)
ARHGAP15	Rho GTPase activating protein 15	4.807	2.186
C9orf152	chromosome 9 open reading frame 152	4.645	1.958
CPA1	carboxypeptidase A1	4.474	1.577
FAM83F	family with sequence similarity 83 member F	4.461	1.694
CDH16	cadherin 16	4.278	1.562
RAB39B	RAB39B, member RAS oncogene family	3.406	1.764
CRHBP	corticotropin releasing hormone binding protein	3.137	1.432
TREM1	triggering receptor expressed on myeloid cells 1	2.908	2.069
TNF	tumor necrosis factor	2.691	1.315
NKX2-3	NK2 homeobox 3	2.690	1.653
BDKRB1	bradykinin receptor B1	2.048	2.227
TUBA8	tubulin alpha 8	1.699	1.324
FNDC11	fibronectin type III domain containing 11	1.698	2.718
IL4I1	interleukin 4 induced 1	1.404	1.449
PPM1J	protein phosphatase, Mg2+/Mn2+ dependent 1J	1.353	1.321
SERPINE1	serpin family E member 1	1.312	15.575
CXCL8	C-X-C motif chemokine ligand 8	1.235	1.890
INPP5D	inositol polyphosphate-5-phosphatase D	1.223	1.567
SIX2	SIX homeobox 2	1.174	2.487
BDKRB2	bradykinin receptor B2	1.133	6.578
TSPAN19	tetraspanin 19	1.094	1.991
ICAM5	intercellular adhesion molecule 5	1.082	2.618
DLG2	discs large MAGUK scaffold protein 2	1.005	1.331
P2RY8	P2Y receptor family member 8	0.976	1.383
TCAF2	TRPM8 channel associated factor 2	0.944	3.036
TNFSF14	TNF superfamily member 14	0.932	1.959
CES1	carboxylesterase 1	0.900	2.513
HSD11B1	hydroxysteroid 11-beta dehydrogenase 1	0.835	1.724
UBB	ubiquitin B	0.825	41.528
ANKRD37	ankyrin repeat domain 37	0.821	3.810
TMEM191C	transmembrane protein 191C	0.791	1.388
SCO2	SCO2, cytochrome c oxidase assembly protein	0.791	1.338
SLC6A9	solute carrier family 6 member 9	0.767	30.239
G0S2	G0/G1 switch 2	0.741	2.227
TRIM36	tripartite motif containing 36	0.738	4.474
CIART	circadian associated repressor of transcription	0.727	5.046
FYN	FYN proto-oncogene, Src family tyrosine kinase	0.704	2.934
CHAC1	ChaC glutathione specific gamma-glutamylcyclotransferase 1	0.697	2.637
TRIB3	tribbles pseudokinase 3	0.688	21.592
SEMA6A	semaphorin 6A	0.685	2.272
GDF15	growth differentiation factor 15	0.683	3.861
HAP1	huntingtin associated protein 1	0.679	2.363
EFNA3	ephrin A3	0.658	1.372
PECAM1	platelet and endothelial cell adhesion molecule 1	0.655	2.512
ABCC6	ATP binding cassette subfamily C member 6	0.643	1.885
PRR19	proline rich 19	0.624	1.558
PPP2R2C	protein phosphatase 2 regulatory subunit Bgamma	0.623	1.630
SERPINB8	serpin family B member 8	0.623	5.933
CD70	CD70 molecule	0.597	2.813
NPIPB3	nuclear pore complex interacting protein family member B3	0.593	3.099
GLI1	GLI family zinc finger 1	0.583	1.324
ZFYVE28	zinc finger FYVE-type containing 28	0.574	3.045
PAK6	p21 (RAC1) activated kinase 6	0.572	1.971
CXCR4	C-X-C motif chemokine receptor 4	0.570	1.612
KRT15	keratin 15	0.569	1.447
NFKBIZ	NFKB inhibitor zeta	0.560	1.709
PHLDA2	pleckstrin homology like domain family A member 2	0.548	13.467
PALM3	paralemmin 3	0.542	1.432
RILP	Rab interacting lysosomal protein	0.536	2.972
CHST2	carbohydrate sulfotransferase 2	0.535	4.291
GLIS3	GLIS family zinc finger 3	0.532	2.027
XKRX	XK related X-linked	0.523	1.323
RELB	RELB proto-oncogene, NF-kB subunit	0.517	2.137
HTR7	5-hydroxytryptamine receptor 7	0.510	4.288
EN2	engrailed homeobox 2	0.507	1.775

SH2D5	SH2 domain containing 5	0.505	1.920
XKR8	XK related 8	0.503	1.467
SLCO4A1	solute carrier organic anion transporter family member 4A1	0.500	9.207
GLRB	glycine receptor beta	-0.502	1.449
GPRASP1	G protein-coupled receptor associated sorting protein 1	-0.504	1.683
NALCN	sodium leak channel, non-selective	-0.511	2.536
SYDE2	synapse defective Rho GTPase homolog 2	-0.516	1.762
RASD2	RASD family member 2	-0.518	1.486
KCNB1	potassium voltage-gated channel subfamily B member 1	-0.518	2.445
RNFT2	ring finger protein, transmembrane 2	-0.530	1.972
MID2	midline 2	-0.535	1.577
C12orf60	chromosome 12 open reading frame 60	-0.538	1.632
GLT8D2	glycosyltransferase 8 domain containing 2	-0.540	3.153
PAPPA	pappalysin 1	-0.541	1.788
LAMA5	laminin subunit alpha 5	-0.550	2.384
SSPO	SCO-spondin	-0.552	2.682
HLA-DMB	major histocompatibility complex, class II, DM beta	-0.553	1.712
GOLGA8A	golgin A8 family member A	-0.555	2.862
GDAP1	ganglioside induced differentiation associated protein 1	-0.557	4.551
NAIP	NLR family apoptosis inhibitory protein	-0.569	1.417
IFI6	interferon alpha inducible protein 6	-0.573	1.667
TSSK4	testis specific serine kinase 4	-0.574	1.482
RNF152	ring finger protein 152	-0.578	4.476
MBLAC1	metallo-beta-lactamase domain containing 1	-0.581	1.563
GUCY1A1	guanylate cyclase 1 soluble subunit alpha 1	-0.581	2.684
CBLN2	cerebellin 2 precursor	-0.595	3.691
C2orf16	chromosome 2 open reading frame 16	-0.606	1.700
HMCN1	hemicentin 1	-0.608	1.337
PGM5	phosphoglucomutase 5	-0.623	1.412
ZFPM2	zinc finger protein, FOG family member 2	-0.627	2.786
HSF2BP	heat shock transcription factor 2 binding protein	-0.628	1.310
NOXA1	NADPH oxidase activator 1	-0.628	1.325
PPL	periplakin	-0.632	2.024
TTN	titin	-0.633	1.994
PPP2R2B	protein phosphatase 2 regulatory subunit Bbeta	-0.636	2.072
JCAD	junctional cadherin 5 associated	-0.641	3.649
METTL27	methyltransferase like 27	-0.645	2.781
EPHA4	EPH receptor A4	-0.652	3.306
GLI2	GLI family zinc finger 2	-0.654	1.749
KRT80	keratin 80	-0.656	3.328
KIT	KIT proto-oncogene receptor tyrosine kinase	-0.659	1.559
EFCAB6	EF-hand calcium binding domain 6	-0.673	2.391
ATOH8	atonal bHLH transcription factor 8	-0.683	1.423
ZNF154	zinc finger protein 154	-0.701	1.459
CSPG4	chondroitin sulfate proteoglycan 4	-0.709	1.893
COLGALT2	collagen beta(1-O)galactosyltransferase 2	-0.726	1.373
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3	-0.733	1.873
PDE5A	phosphodiesterase 5A	-0.744	4.560
GPT	glutamic--pyruvic transaminase	-0.802	4.589
EGR3	early growth response 3	-0.809	1.421
C1QTNF2	C1q and TNF related 2	-0.814	1.623
MOV10L1	Mov10 like RISC complex RNA helicase 1	-0.830	3.639
ZSCAN23	zinc finger and SCAN domain containing 23	-0.832	1.536
EPB41L4B	erythrocyte membrane protein band 4.1 like 4B	-0.839	1.364
GOLGA8N	golgin A8 family member N	-0.840	2.326
ABI3BP	ABI family member 3 binding protein	-0.854	1.481
CTTNBP2	cortactin binding protein 2	-0.893	6.170
TJP2	tight junction protein 2	-0.919	1.606
IGSF9B	immunoglobulin superfamily member 9B	-0.970	4.234
EGR2	early growth response 2	-0.979	1.452
NAP1L2	nucleosome assembly protein 1 like 2	-1.015	1.472
CRISPLD1	cysteine rich secretory protein LCCL domain containing 1	-1.022	2.115
TUBA3D	tubulin alpha 3d	-1.038	1.757
C4A	complement C4A (Rodgers blood group)	-1.038	2.369
AC092718.8	novel protein	-1.144	1.533
ENTPD8	ectonucleoside triphosphate diphosphohydrolase 8	-1.271	2.076
SLC4A4	solute carrier family 4 member 4	-1.301	1.434
POSTN	periostin	-1.302	2.312
ALPK3	alpha kinase 3	-1.342	1.640
PI15	peptidase inhibitor 15	-1.348	4.241
AC119396.1	novel protein	-1.357	1.321

CFAP57	cilia and flagella associated protein 57	-1.399	1.433
SPANXD	SPANX family member D	-1.423	1.374
PTGER2	prostaglandin E receptor 2	-1.429	2.793
GALNTL6	polypeptide N-acetylgalactosaminyltransferase like 6	-1.431	1.511
NTN3	netrin 3	-1.442	1.443
AC015802.6	novel protein	-1.470	1.726
AC233992.2	novel transcript	-1.551	1.493
XDH	xanthine dehydrogenase	-1.578	1.482
TEX52	testis expressed 52	-1.581	2.121
IFI27	interferon alpha inducible protein 27	-1.641	2.426
SPATA9	spermatogenesis associated 9	-1.697	1.782
NUDT7	nudix hydrolase 7	-1.699	2.222
RNF150	ring finger protein 150	-1.739	3.840
CXCR6	C-X-C motif chemokine receptor 6	-1.770	1.586
METTL7B	methyltransferase like 7B	-1.799	1.363
FSTL4	follistatin like 4	-1.838	1.364
FBLN2	fibulin 2	-1.887	1.400
ZPLD1	zona pellucida like domain containing 1	-1.905	1.452
CYFIP2	cytoplasmic FMR1 interacting protein 2	-2.061	1.390
APELA	apelin receptor early endogenous ligand	-2.148	1.767
ACMSD	aminocarboxymuconate semialdehyde decarboxylase	-2.265	1.395
IZUMO1	izumo sperm-egg fusion 1	-2.306	1.445
STPG1	sperm tail PG-rich repeat containing 1	-2.366	1.743
MMP12	matrix metallopeptidase 12	-2.698	1.331
EGR1	early growth response 1	-2.825	3.705
NFE4	nuclear factor, erythroid 4	-3.019	1.366
SENP3-EIF4A1	SENP3-EIF4A1 readthrough (NMD candidate)	-3.614	1.948
LRRC43	leucine rich repeat containing 43	-3.615	1.424
ATP6V1G3	ATPase H+ transporting V1 subunit G3	-3.731	1.529
TBC1D3K	TBC1 domain family member 3K	-3.818	1.391
AL136531.2	novel transcript	-3.828	1.625
LPAR5	lysophosphatidic acid receptor 5	-3.888	1.743
KLRC4-KLRK1	KLRC4-KLRK1 readthrough	-3.888	1.740
ACTG2	actin, gamma 2, smooth muscle, enteric	-3.989	1.792
ASPDH	aspartate dehydrogenase domain containing	-4.067	1.390
ABCB1	ATP binding cassette subfamily B member 1	-4.091	1.428
AL358472.6	novel protein	-4.101	1.381
KLRC4	killer cell lectin like receptor C4	-4.469	1.860
FAM71D	family with sequence similarity 71 member D	-4.642	1.949
TMEM179	transmembrane protein 179	-4.899	2.096

Table S2. Effects of Simvastatin on differential gene expression in LPS-stimulated T98G cells. Only protein coding genes with log_2fold change > |0.5| that were selectively altered by Simvastatin in nt siRNA treated cells but not altered in Nurr1 silenced cells are shown.

Gene name	Gene description	log_2 (fold change)	$-log_{10}$ (p value)
HK3	hexokinase 3	4.559	2.002
GJB5	gap junction protein beta 5	4.250	1.605
C3orf67	chromosome 3 open reading frame 67	4.242	2.106
LRRD1	leucine rich repeats and death domain containing 1	4.195	2.135
CLEC18A	C-type lectin domain family 18 member A	4.083	1.379
CFHR1	complement factor H related 1	4.083	1.439
KLHL35	kelch like family member 35	4.080	1.397
COL9A2	collagen type IX alpha 2 chain	4.070	1.368
RNF43	ring finger protein 43	4.004	1.785
RNF222	ring finger protein 222	3.708	1.545
C16orf46	chromosome 16 open reading frame 46	3.367	1.431
MUC15	mucin 15, cell surface associated	2.549	1.302
RCAN2	regulator of calcineurin 2	1.823	2.312
ATP10B	ATPase phospholipid transporting 10B (putative)	1.761	1.744
DNAJB13	DnaJ heat shock protein family (Hsp40) member B13	1.752	1.835
C11orf96	chromosome 11 open reading frame 96	1.704	3.066
PHOSPHO1	phosphoethanolamine/phosphocholine phosphatase	1.339	1.303
SNAI3	snail family transcriptional repressor 3	1.124	3.420
TMEM216	transmembrane protein 216	1.116	2.046
NOTCH4	notch 4	1.020	1.821
EPS8L1	EPS8 like 1	1.019	1.896
C19orf73	chromosome 19 open reading frame 73	0.842	1.828
AKAP12	A-kinase anchoring protein 12	0.837	1.637
XKRX	XK related X-linked	0.831	2.609
CCDC151	coiled-coil domain containing 151	0.816	1.625
PTGS2	prostaglandin-endoperoxide synthase 2	0.789	8.047
FKBP1B	FK506 binding protein 1B	0.781	1.336
DNAAF3	dynein axonemal assembly factor 3	0.778	2.180
INHBE	inhibin subunit beta E	0.771	1.551
CPEB3	cytoplasmic polyadenylation element binding protein 3	0.749	1.484
HSD11B1	hydroxysteroid 11-beta dehydrogenase 1	0.738	1.525
CD7	CD7 molecule	0.713	2.130
SLC2A5	solute carrier family 2 member 5	0.688	1.390
C4A	complement C4A (Rodgers blood group)	0.685	1.540
ABCA3	ATP binding cassette subfamily A member 3	0.680	1.863
ICAM1	intercellular adhesion molecule 1	0.675	4.838
STBD1	starch binding domain 1	0.640	1.932
EN2	engrailed homeobox 2	0.638	3.077
SWSAP1	SWIM-type zinc finger 7 associated protein 1	0.632	1.534
CDKN2D	cyclin dependent kinase inhibitor 2D	0.631	6.371
ATP6V0C	ATPase H+ transporting V0 subunit c	0.618	2.651
TMPRSS6	transmembrane serine protease 6	0.610	1.352
PAK6	p21 (RAC1) activated kinase 6	0.602	1.844
TUBB2A	tubulin beta 2A class IIa	0.600	6.127
SERPINE1	serpin family E member 1	0.599	4.268
MT1X	metallothionein 1X	0.547	4.060
TRNP1	TMF1-regulated nuclear protein 1	0.542	2.065
ETV4	ETS variant 4	0.538	12.089
SEMA6A	semaphorin 6A	0.512	1.731
CBLN2	cerebellin 2 precursor	-0.502	2.847
DENND2A	DENN domain containing 2A	-0.506	1.386
KIAA0319	KIAA0319	-0.515	1.774

TLN2	talin 2	-0.516	4.222
IL31RA	interleukin 31 receptor A	-0.517	3.058
LEAP2	liver enriched antimicrobial peptide 2	-0.526	1.638
LDLRAD4	low density lipoprotein receptor class A domain containing 4	-0.529	1.761
S100A1	S100 calcium binding protein A1	-0.539	1.517
GABRA3	gamma-aminobutyric acid type A receptor alpha3 subunit	-0.555	2.330
GATA2	GATA binding protein 2	-0.559	1.589
KCNB1	potassium voltage-gated channel subfamily B member 1	-0.571	3.349
TRPV2	transient receptor potential cation channel subfamily V member 2	-0.580	1.503
PSG4	pregnancy specific beta-1-glycoprotein 4	-0.588	1.799
NPIPB9	nuclear pore complex interacting protein family member B9	-0.616	1.306
ZNF835	zinc finger protein 835	-0.617	1.418
ANKRD23	ankyrin repeat domain 23	-0.640	1.324
ZNF10	zinc finger protein 10	-0.641	2.733
CKMT2	creatine kinase, mitochondrial 2	-0.729	1.788
TNXB	tenascin XB	-0.741	1.565
MYH3	myosin heavy chain 3	-0.772	1.930
FGF14	fibroblast growth factor 14	-0.789	1.989
COX16	cytochrome c oxidase assembly factor COX16	-0.808	1.951
PI15	peptidase inhibitor 15	-0.811	2.124
TSTD3	thiosulfate sulfurtransferase like domain containing 3	-0.832	1.303
AKAP5	A-kinase anchoring protein 5	-0.847	1.468
SLC16A14	solute carrier family 16 member 14	-0.855	1.412
FHOD3	formin homology 2 domain containing 3	-0.860	1.560
AC009336.2	homeobox D4	-0.918	1.433
CDRT15	CMT1A duplicated region transcript 15	-1.043	1.479
KCNA7	potassium voltage-gated channel subfamily A member 7	-1.093	1.686
TYRP1	tyrosinase related protein 1	-1.165	2.589
FBXO24	F-box protein 24	-1.201	1.965
DNAH7	dynein axonemal heavy chain 7	-1.204	1.679
CPA5	carboxypeptidase A5	-1.210	1.618
MARCH4	membrane associated ring-CH-type finger 4	-1.219	1.886
SLC35G6	solute carrier family 35 member G6	-1.249	1.402
C3orf80	chromosome 3 open reading frame 80	-1.420	1.404
PROSER2	proline and serine rich 2	-1.728	2.285
PDE6B	phosphodiesterase 6B	-1.752	1.471
METTL7B	methyltransferase like 7B	-1.838	1.388
BEGAIN	brain enriched guanylate kinase associated	-1.841	2.021
GRM4	glutamate metabotropic receptor 4	-2.086	1.341
RAB11FIP1	RAB11 family interacting protein 1	-2.102	1.743
APELA	apelin receptor early endogenous ligand	-2.210	1.466
DEF6	DEF6, guanine nucleotide exchange factor	-2.264	2.100
NANOS3	nanos C2HC-type zinc finger 3	-2.471	1.420
MYBPC1	myosin binding protein C, slow type	-2.520	1.808
ACSL6	acyl-CoA synthetase long chain family member 6	-2.891	1.999
ANKRD65	ankyrin repeat domain 65	-3.200	1.496
GPR83	G protein-coupled receptor 83	-3.808	1.638
DEGS2	delta 4-desaturase, sphingolipid 2	-3.882	1.673
SLC6A12	solute carrier family 6 member 12	-4.119	1.445
VSTM5	V-set and transmembrane domain containing 5	-4.238	1.512
HORMAD1	HORMA domain containing 1	-4.282	1.606
GSDMC	gasdermin C	-4.357	1.454
SLC35F1	solute carrier family 35 member F1	-4.426	2.395
EBF3	early B cell factor 3	-4.531	1.889
HS6ST2	heparan sulfate 6-O-sulfotransferase 2	-4.656	2.089
ZNF366	zinc finger protein 366	-4.718	2.046
TBC1D3B	TBC1 domain family member 3B	-7.221	2.770

Supporting Methods

Computational Methods.

General: Calculations were conducted in KNIME (version 3.7.2, KNIME AG, Zurich, Switzerland) and Molecular Operating Environment (MOE, version 2018.0101, Chemical Computing Group Inc. Montreal, QC, Canada) using default settings for each tool/function unless stated otherwise. Amber10:EHT was used as default force field for all calculations. *Library processing*: Analysis of the drug fragment library from Prestwick (Prestwick Chemical, Illkirch, France) was performed in KNIME using the provided SMILES strings compared to the DrugBank database (all drug structures in SDF Format, version 5.1.1, released on 2018-07-03). The RDKit extension nodes (version 4.0.1.v202002121354) were used to filter for PAINs structures and calculate features (MW, clogP, number of H-bond donors/acceptors and rotatable bonds, aromatic rings, TPSA). For hits from the primary screen, a search for parent and related drugs was performed via molecule substructure and murcko scaffold (both RDKit nodes) compared to the DrugBank database (version 5.1.1). Graph based frameworks were extracted with the MOE KNIME extension node murcko frameworks ignoring small terminal rings of size 3 or 4. Rings of size 5 to 7 atoms as well as annealed rings, bicyclo and spiro compounds of equal ring count were assigned to the same groups. Geometry in terms of linker attachment points and connectivity was ignored, only the linker length was considered. *Multiple alignment*: Molecular structures of amodiaquine, fluvastatin and pitavastatin were prepared using MOE Wash tool: protonation state dominant at pH 7; coordinates rebuild 3D; preserved existing chirality. Multiple alignment of these three compounds was performed using default settings from MOE flexible alignment tool.

Supporting References

[1] D. Zaienne, S. Willems, S. Schierle, J. Heering, D. Merk, *J. Med. Chem.* **2021**, *64*, 15126–15140.