Supplementary Table 1: List of 490 proteins. A list of proteins of interest in PCa with UniProtID, Symbol, EntrezId and Description.

Uniprot ID	Entry name	Protein names	Official gene name
O00170	AIP_HUMAN	AH receptor-interacting protein	AIP
O00763	ACACB_HUMAN	Acetyl-CoA carboxylase 2	ACACB
O94788	AL1A2_HUMAN	Retinal dehydrogenase 2	ALDH1A2
P01116	RASK_HUMAN	GTPase KRas	KRAS
P06730	IF4E_HUMAN	Eukaryotic translation initiation factor 4E	EIF4E
P07288	KLK3_HUMAN	Prostate-specific antigen	KLK3
P07900	HS90A_HUMAN	Heat shock protein HSP 90-alpha	HSP90AA1
P07951	TPM2_HUMAN	Tropomyosin beta chain	TPM2
P09874	PARP1_HUMAN	Poly [ADP-ribose] polymerase 1	PARP1
P12830	CADH1_HUMAN	Cadherin-1	CDH1
P12931	SRC_HUMAN	Proto-oncogene tyrosine-protein kinase Src	SRC
P16949	STMN1_HUMAN	Stathmin	STMN1
P19838	NFKB1_HUMAN	Nuclear factor NF-kappa-B p105 subunit	NFKB1
P20151	KLK2_HUMAN	Kallikrein-2	KLK2
P20700	LMNB1_HUMAN	Lamin-B1	LMNB1
P24844	MYL9_HUMAN	Myosin regulatory light polypeptide 9	MYL9
P26358	DNMT1_HUMAN	DNA (cytosine-5)-methyltransferase 1	DNMT1
P27797	CALR_HUMAN	Calreticulin	CALR
P28482	MK01_HUMAN	Mitogen-activated protein kinase 1	MAPK1
P30086	PEBP1_HUMAN	Phosphatidylethanolamine-binding protein 1	PEBP1
P31946	1433B_HUMAN	14-3-3 protein beta/alpha	YWHAB
P33991	MCM4_HUMAN	DNA replication licensing factor MCM4	MCM4
P35221	CTNA1_HUMAN	Catenin alpha-1	CTNNA1
P35222	CTNB1_HUMAN	Catenin beta-1	CTNNB1
P35749	MYH11_HUMAN	Myosin-11	MYH11
P36507	MP2K2_HUMAN	Dual specificity mitogen-activated protein kinase kinase 2	MAP2K2
P40763	STAT3_HUMAN	Signal transducer and activator of transcription 3	STAT3
P42224	STAT1_HUMAN	Signal transducer and activator of transcription 1-alpha/beta	STAT1
P49327	FAS_HUMAN	Fatty acid synthase	FASN
P51532	SMCA4_HUMAN	Transcription activator BRG1	SMARCA4
P60763	RAC3_HUMAN	Ras-related C3 botulinum toxin substrate 3	RAC3
P60953	CDC42_HUMAN	Cell division control protein 42 homolog	CDC42
P61158	ARP3_HUMAN	Actin-related protein 3	ACTR3
P61160	ARP2_HUMAN	Actin-related protein 2	ACTR2
P61586	RHOA_HUMAN	Transforming protein RhoA	RHOA
P62258	1433E_HUMAN	14-3-3 protein epsilon	YWHAE
P62736	ACTA_HUMAN	Actin, aortic smooth muscle	ACTA2
P62826	RAN_HUMAN	GTP-binding nuclear protein Ran	RAN
P63104	1433Z_HUMAN	14-3-3 protein zeta/delta	YWHAZ
P99999	CYC_HUMAN	Cytochrome c	CYCS
Q02750	MP2K1_HUMAN	Dual specificity mitogen-activated protein kinase kinase 1	MAP2K1

Q06124	PTN11_HUMAN	Tyrosine-protein phosphatase non-receptor type 11	PTPN11
Q13085	ACACA_HUMAN	Acetyl-CoA carboxylase 1	ACACA
Q13153	PAK1_HUMAN	Serine/threonine-protein kinase PAK 1	PAK1
Q13451	FKBP5_HUMAN	Peptidyl-prolyl cis-trans isomerase FKBP5	FKBP5
Q13480	GAB1_HUMAN	GRB2-associated-binding protein 1	GAB1
Q15392	DHC24_HUMAN	Delta(24)-sterol reductase	DHCR24
Q16543	CDC37_HUMAN	Hsp90 co-chaperone Cdc37	CDC37
Q8N0X7	SPART_HUMAN	Spartin	SPART
Q96R06	SPAG5_HUMAN	Sperm-associated antigen 5	SPAG5
Q9UK76	JUPI1_HUMAN	Jupiter microtubule associated homolog 1	JPT1
Q9UQ80	PA2G4_HUMAN	Proliferation-associated protein 2G4	PA2G4

Supplementary Table 2: Pathway enrichment analysis. All quantified 52 proteins were analysed using REACTOME pathway enrichment analysis tool REACTOME. The enriched pathways are listed together with their respective statistical significance values.

Pathway identifier	Pathway name	Entities pValue	Entities FDR
R-HSA-195258	RHO GTPase Effectors	5.27E-14	3.70E-11
R-HSA-194315	Signaling by Rho GTPases	1.78E-12	6.24E-10
R-HSA-9006934	Signaling by Receptor Tyrosine Kinases	1.13E-11	2.64E-09
R-HSA-5625740	RHO GTPases activate PKNs	4.53E-11	7.92E-09
R-HSA-162582	Signal Transduction	5.33E-10	7.46E-08
R-HSA-6802948	Signaling by high-kinase activity BRAF mutants	9.01E-10	9.67E-08
R-HSA-5663202	Diseases of signal transduction	9.67E-10	9.67E-08
R-HSA-5674135	MAP2K and MAPK activation	1.86E-09	1.30E-07
R-HSA-6802946	Signaling by moderate kinase activity BRAF mutants	1.86E-09	1.30E-07
R-HSA-6802955	Paradoxical activation of RAF signaling by kinase inactive BRAF	1.86E-09	1.30E-07
R-HSA-6802949	Signaling by RAS mutants	1.26E-08	7.96E-07
R-HSA-422475	Axon guidance	1.41E-08	8.17E-07
R-HSA-1643685	Disease	2.12E-08	1.13E-06
R-HSA-2682334	EPH-Ephrin signaling	2.68E-08	1.34E-06
R-HSA-6802952	Signaling by BRAF and RAF fusions	2.93E-08	1.35E-06
R-HSA-4420097	VEGFA-VEGFR2 Pathway	4.01E-08	1.73E-06
R-HSA-5675221	Negative regulation of MAPK pathway	7.03E-08	2.88E-06
R-HSA-194138	Signaling by VEGF	7.88E-08	2.99E-06
R-HSA-3928662	EPHB-mediated forward signaling	9.34E-08	3.36E-06
R-HSA-1433557	Signaling by SCF-KIT	1.23E-07	4.27E-06
R-HSA-1266738	Developmental Biology	1.29E-07	4.27E-06
R-HSA-6802957	Oncogenic MAPK signaling	1.44E-07	4.46E-06
R-HSA-5673000	RAF activation	2.27E-07	6.80E-06
R-HSA-1227986	Signaling by ERBB2	4.47E-07	1.30E-05
R-HSA-449147	Signaling by Interleukins	4.75E-07	1.33E-05
R-HSA-186763	Downstream signal transduction	5.53E-07	1.44E-05
R-HSA-445355	Smooth Muscle Contraction	1.17E-06	2.93E-05
R-HSA-5637810	Constitutive Signaling by EGFRvIII	1.27E-06	2.93E-05
R-HSA-5637812	Signaling by EGFRvIII in Cancer	1.27E-06	2.93E-05
R-HSA-1059683	Interleukin-6 signaling	1.27E-06	2.93E-05
R-HSA-5654743	Signaling by FGFR4	2.84E-06	5.81E-05
R-HSA-5654741	Signaling by FGFR3	2.84E-06	5.81E-05
R-HSA-5683057	MAPK family signaling cascades	2.87E-06	5.81E-05
R-HSA-177929	Signaling by EGFR	3.18E-06	5.81E-05
R-HSA-5637815	Signaling by Ligand-Responsive EGFR Variants in Cancer	3.23E-06	5.81E-05
R-HSA-1236382	Constitutive Signaling by Ligand-Responsive EGFR Cancer Variants	3.23E-06	5.81E-05

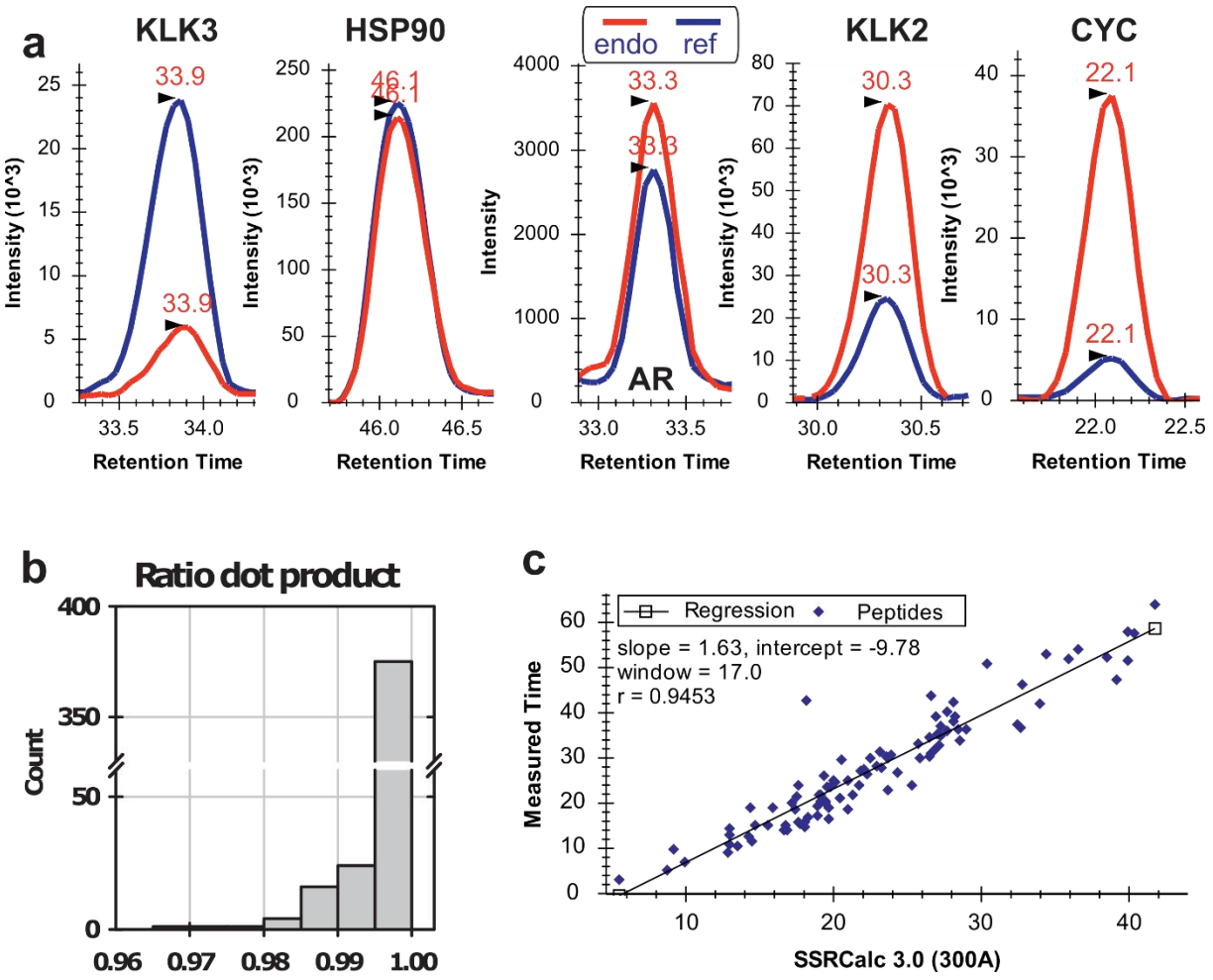
R-HSA-1643713	Signaling by EGFR in Cancer	3.23E-06	5.81E-05
R-HSA-5627117	RHO GTPases Activate ROCKs	3.23E-06	5.81E-05
R-HSA-187037	NGF signalling via TRKA from the plasma membrane	3.57E-06	6.07E-05
R-HSA-166520	Signalling by NGF	3.74E-06	6.37E-05
R-HSA-5674499	Negative feedback regulation of MAPK pathway	4.60E-06	7.65E-05
R-HSA-5627123	RHO GTPases activate PAKs	4.78E-06	7.65E-05
R-HSA-445144	Signal transduction by L1	4.78E-06	7.65E-05
R-HSA-1280215	Cytokine Signaling in Immune system	6.12E-06	9.18E-05
R-HSA-112409	RAF-independent MAPK1/3 activation	8.06E-06	1.21E-04
R-HSA-5654736	Signaling by FGFR1	8.69E-06	1.30E-04
R-HSA-163765	ChREBP activates metabolic gene expression	1.08E-05	1.52E-04
R-HSA-447115	Interleukin-12 family signaling	1.23E-05	1.72E-04
R-HSA-5218920	VEGFR2 mediated vascular permeability	1.28E-05	1.79E-04
R-HSA-168256	Immune System	1.36E-05	1.89E-04
R-HSA-186797	Signaling by PDGF	1.45E-05	1.89E-04
R-HSA-6783589	Interleukin-6 family signaling	1.47E-05	1.91E-04
R-HSA-3928663	EPHA-mediated growth cone collapse	1.69E-05	2.19E-04
R-HSA-8876493	InlA-mediated entry of Listeria monocytogenes into host cells	2.10E-05	2.52E-04
R-HSA-112411	MAPK1 (ERK2) activation	2.10E-05	2.52E-04
R-HSA-373755	Semaphorin interactions	2.14E-05	2.57E-04
R-HSA-5626467	RHO GTPases activate IQGAPs	2.18E-05	2.62E-04
R-HSA-109581	Apoptosis	2.77E-05	3.06E-04
R-HSA-180292	GAB1 signalosome	2.78E-05	3.06E-04
R-HSA-2029480	Fcgamma receptor (FCGR) dependent phagocytosis	3.33E-05	3.66E-04
R-HSA-5357801	Programmed Cell Death	3.33E-05	3.66E-04
R-HSA-5663213	RHO GTPases Activate WASPs and WAVEs	3.90E-05	4.29E-04
R-HSA-75035	Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex	4.56E-05	5.02E-04
R-HSA-5654738	Signaling by FGFR2	5.12E-05	5.12E-04
R-HSA-5684996	MAPK1/MAPK3 signaling	5.60E-05	5.60E-04
R-HSA-6806834	Signaling by MET	5.78E-05	5.78E-04
R-HSA-5655302	Signaling by FGFR1 in disease	5.85E-05	5.85E-04
R-HSA-111447	Activation of BAD and translocation to mitochondria	6.96E-05	6.96E-04
R-HSA-109606	Intrinsic Pathway for Apoptosis	7.72E-05	7.57E-04
R-HSA-1295596	Spry regulation of FGF signaling	8.41E-05	7.57E-04

Supplementary Table 3: Skyline output of perturbation matrix. All transitions quantified as function of treatment condition. This table served as input for MSStats.

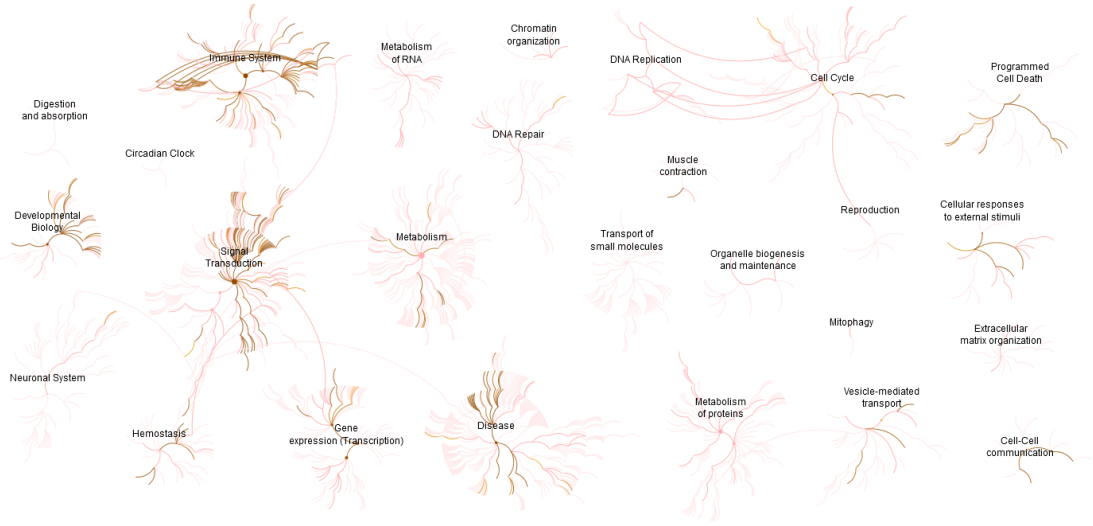
Supplementary Table 3 = separate csv file

Supplementary Table 4: Copy number alterations for cell lines. The cBioPortal was queried for DNA copy number alterations of YWHAZ, MYC and NCOA2 of all available cell line models. Prostate cancer models are highlighted with yellow background.

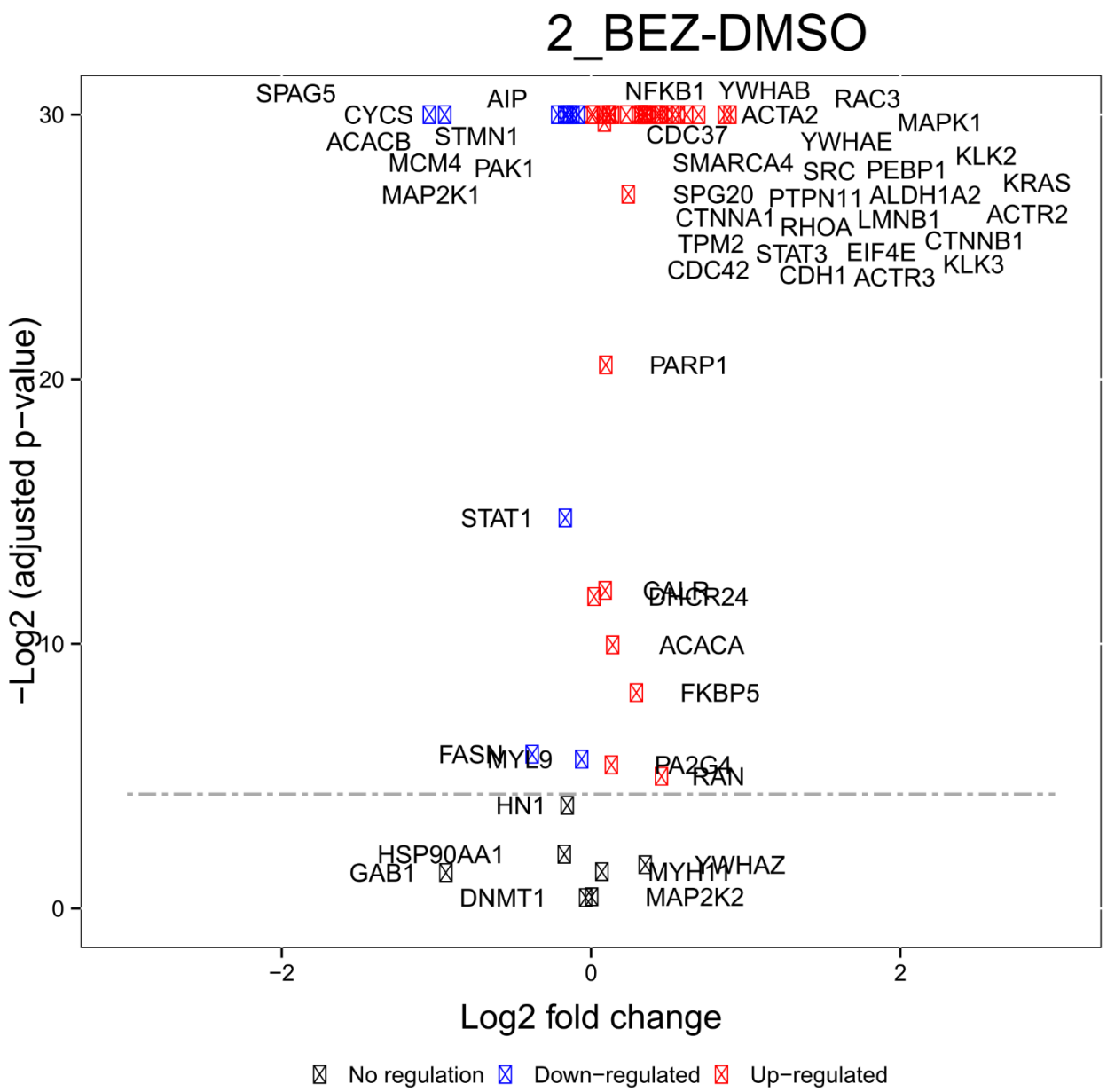
Supplementary Table 4 = separate csv file



Supplementary Figure 1: SRM assay development. Panel a: Ion chromatograms of the sum of all transitions per peptide which are base line separated from other signals measured. In blue lines the sum of all transition of SILAC labelled peptides while in red are ion chromatograms of endogenous unlabelled peptides isolated from LNCaP cells. Both blue and red lines co-elute which adds further evidence for high confidence SRM assays. Protein names given are official gene names. **Panel b**: The ratio-dot-product is a mathematical description of how similar both SILAC labelled and non-labelled peptide fragments elute, e.g. relative ratio of transitions measured. A score of 1 means identical pattern in both SILAC and non-labelled transitions. The great majority of peptides have a ratio-dot-products of greater than 0.99 indicative of high quality SRM assays. **Panel c**: Measured retention time is plotted as a function of theoretically calculated retention time SSRCalc 3.0 (as function of primary amino acid sequence of the peptide). A linear regression line has a very good r value (r = 0.95) further supporting the notion of high quality SRM assays.



Supplementary Figure 2: Pathway enrichment analysis: visualization. All quantified 52 proteins were analysed using RACTOME pathway enrichment analysis tool REACTOME. A visual representation of the results shows a large coverage of many pathways, e.g. programmed cell death, signal transduction and immune system.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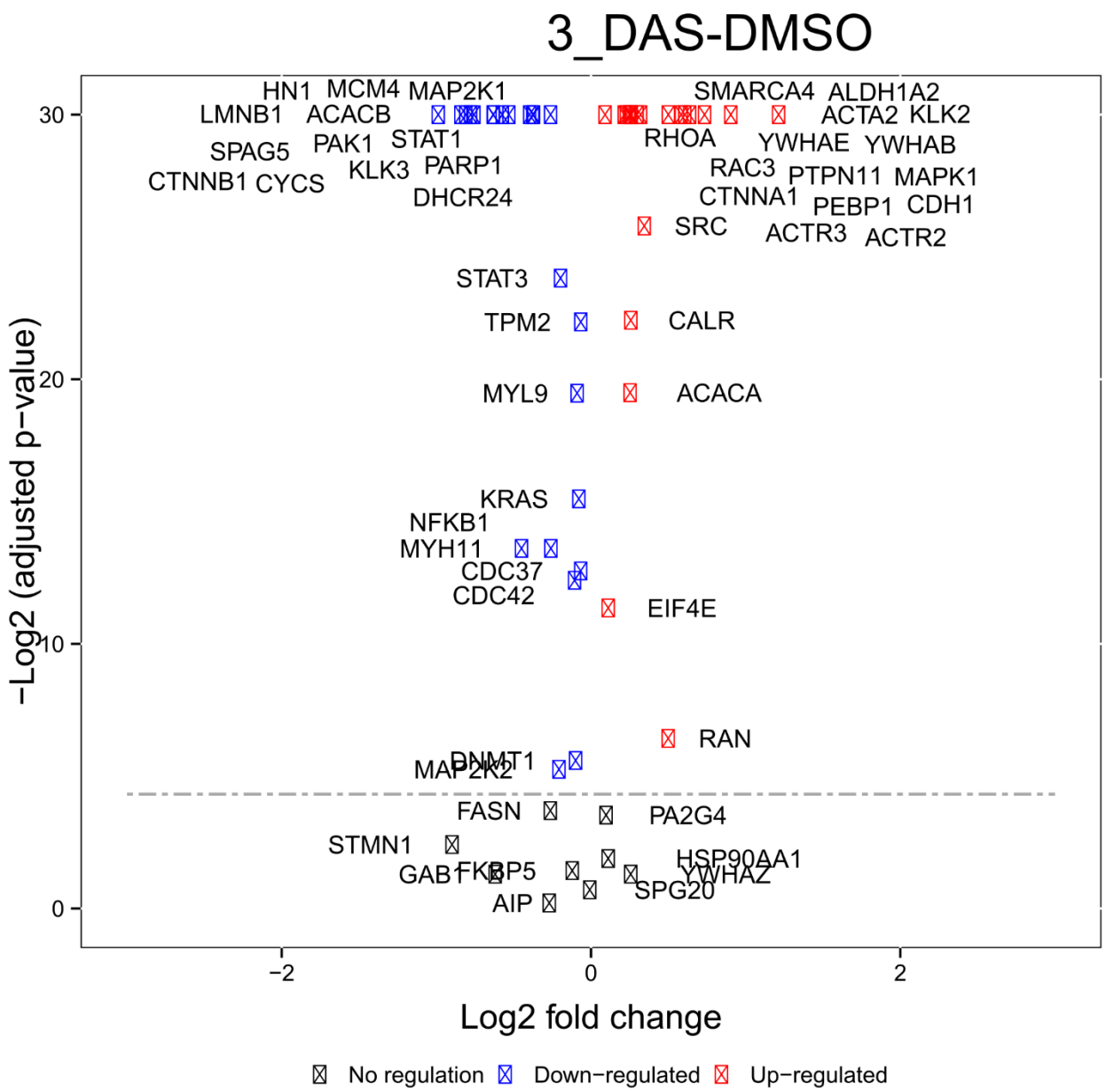
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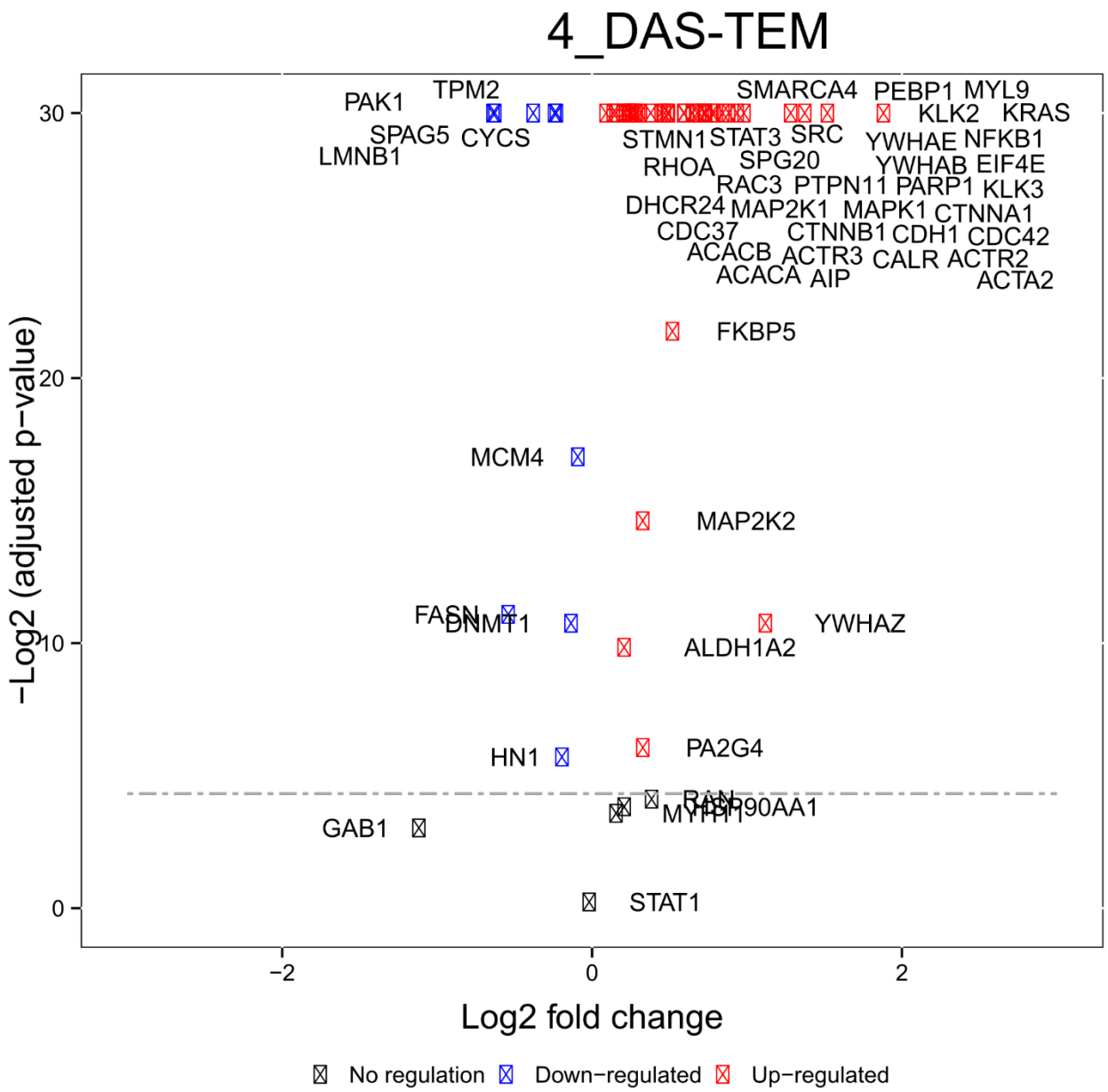
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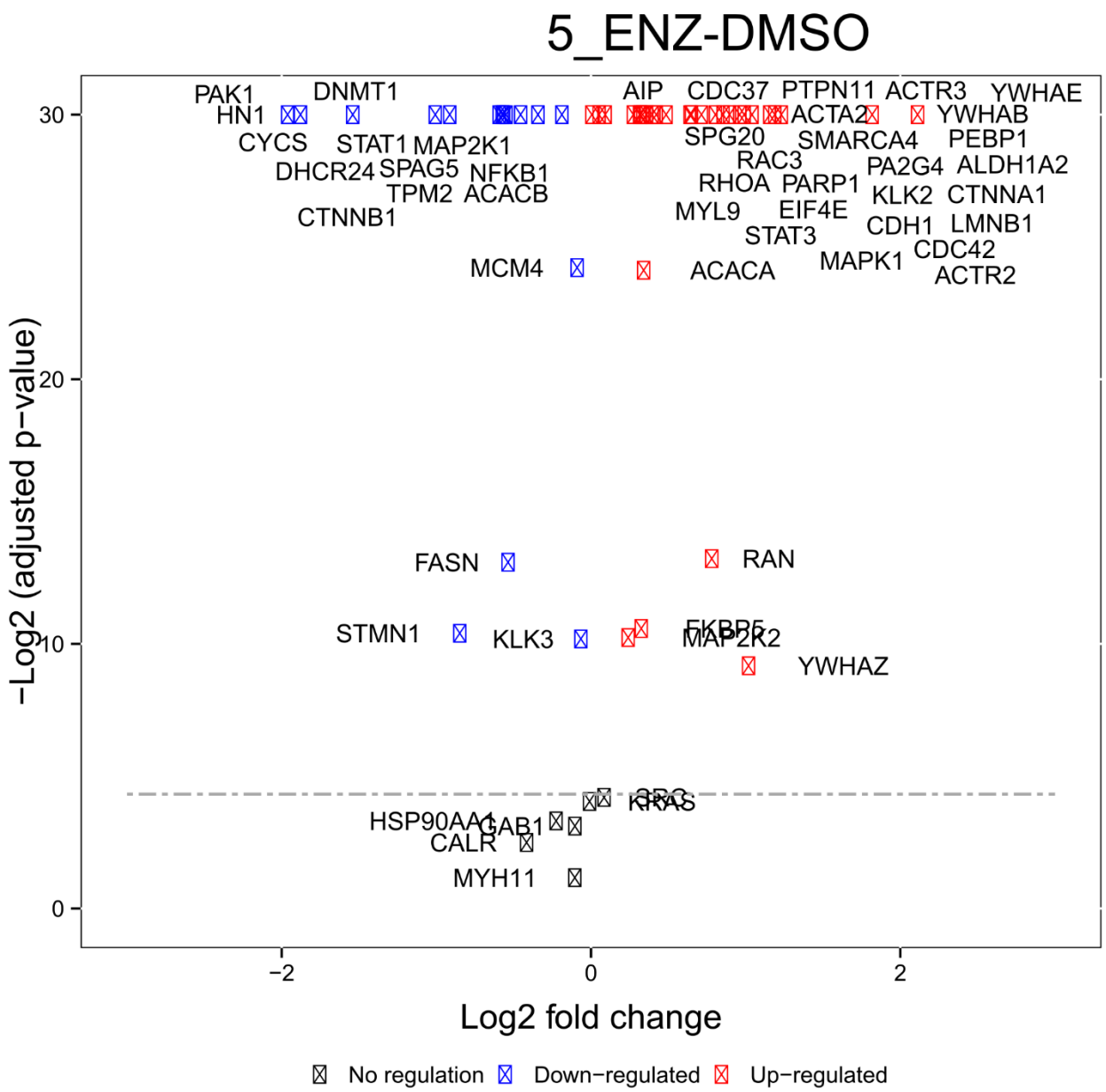
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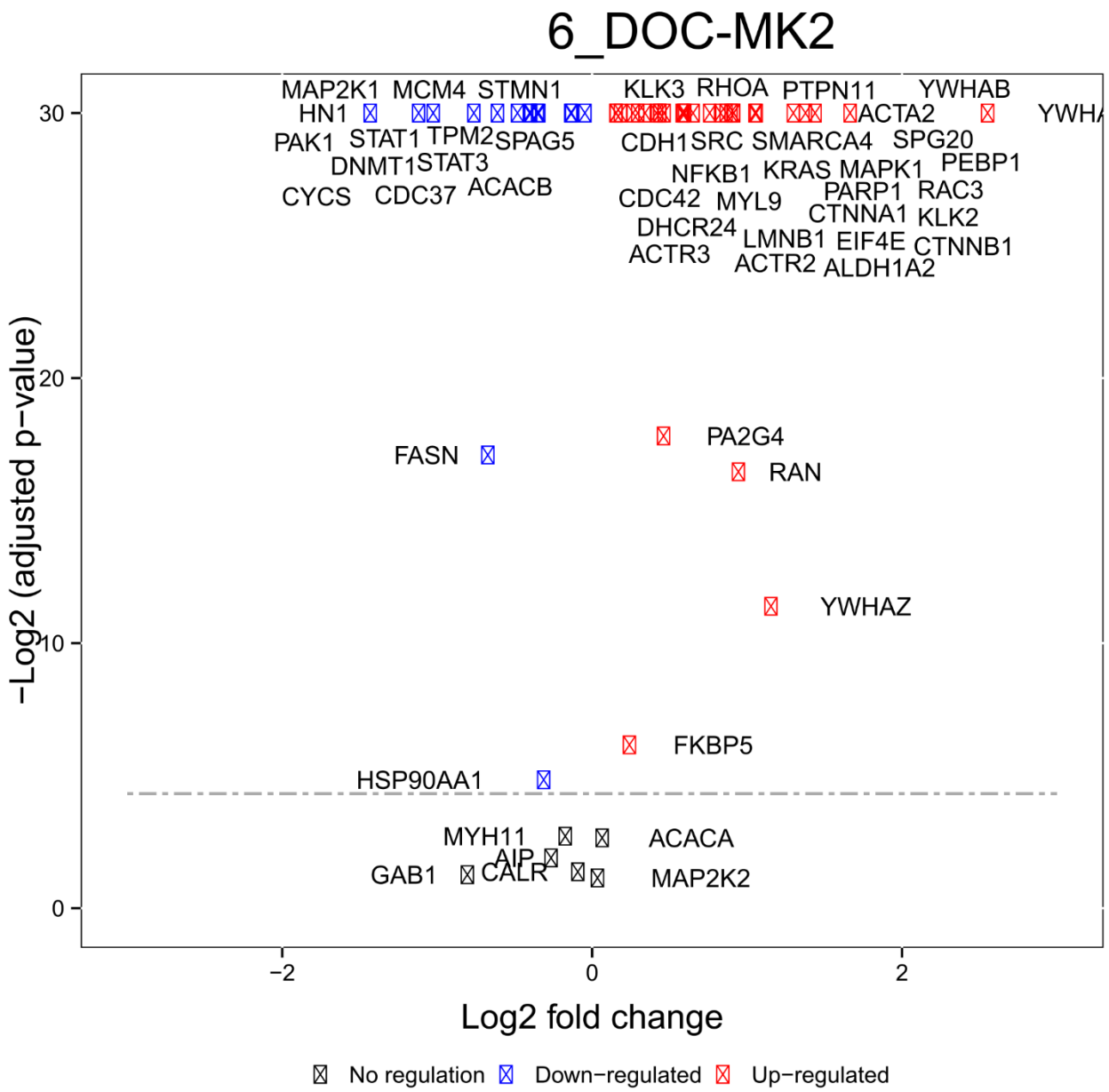
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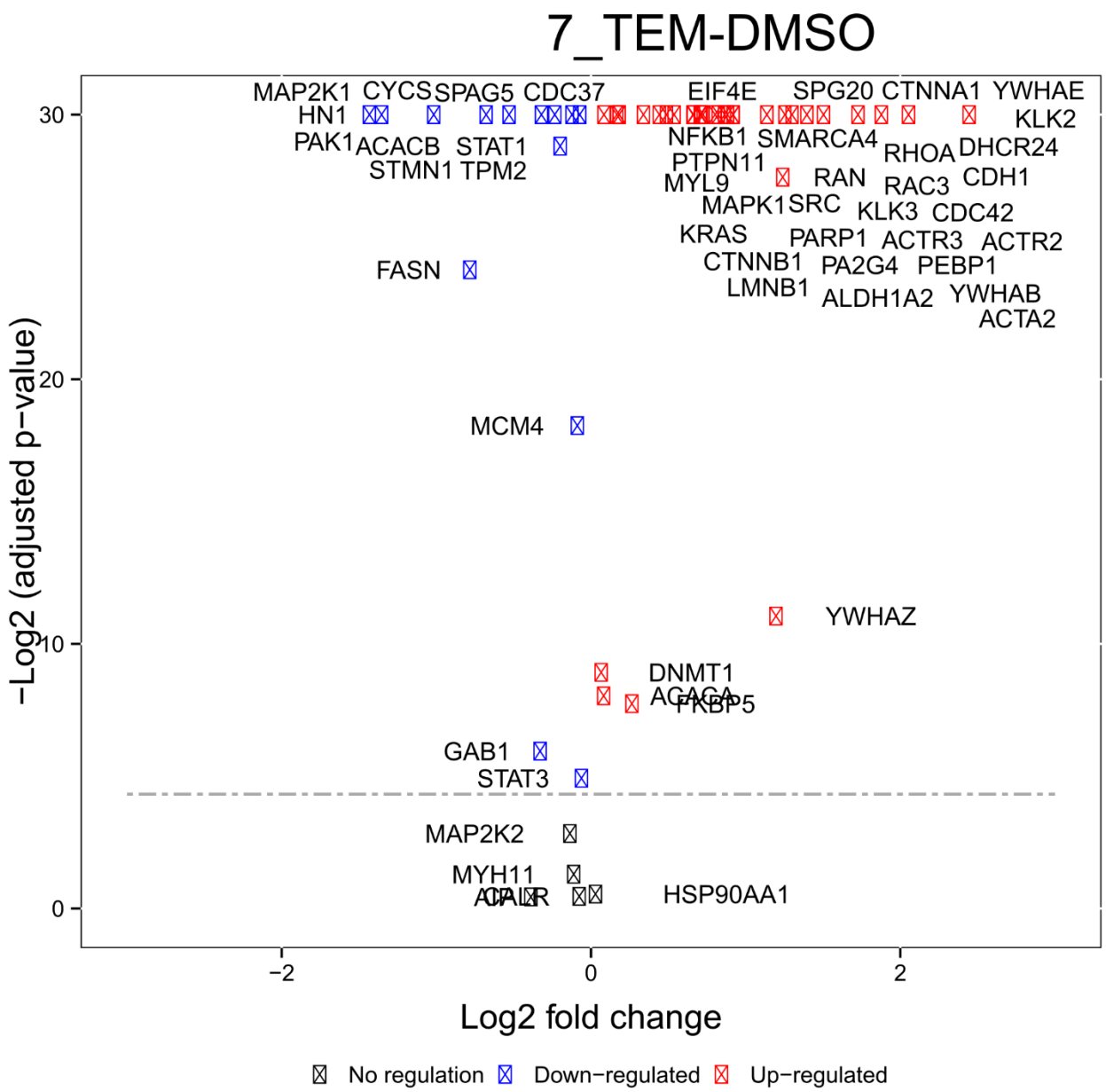
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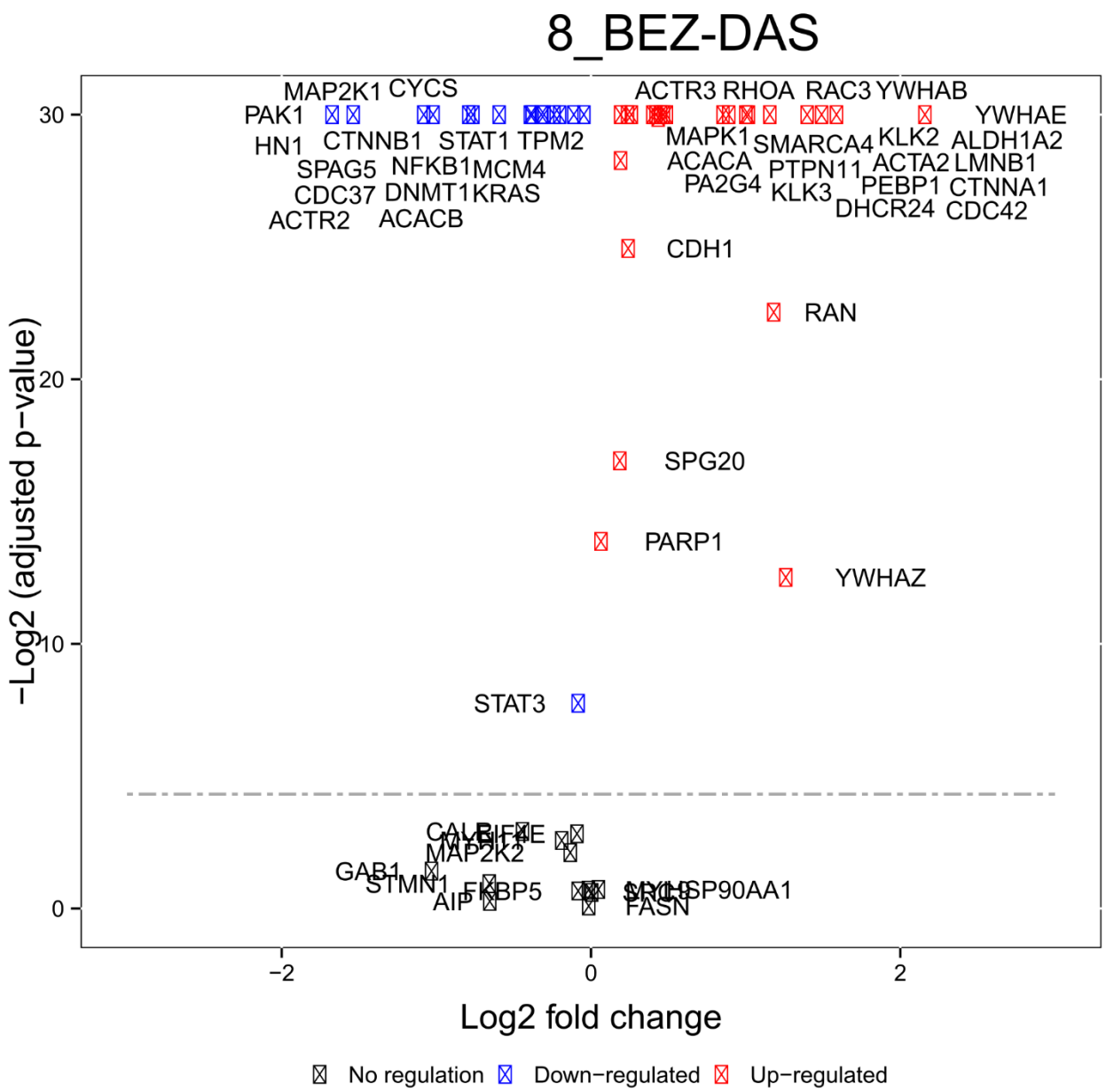
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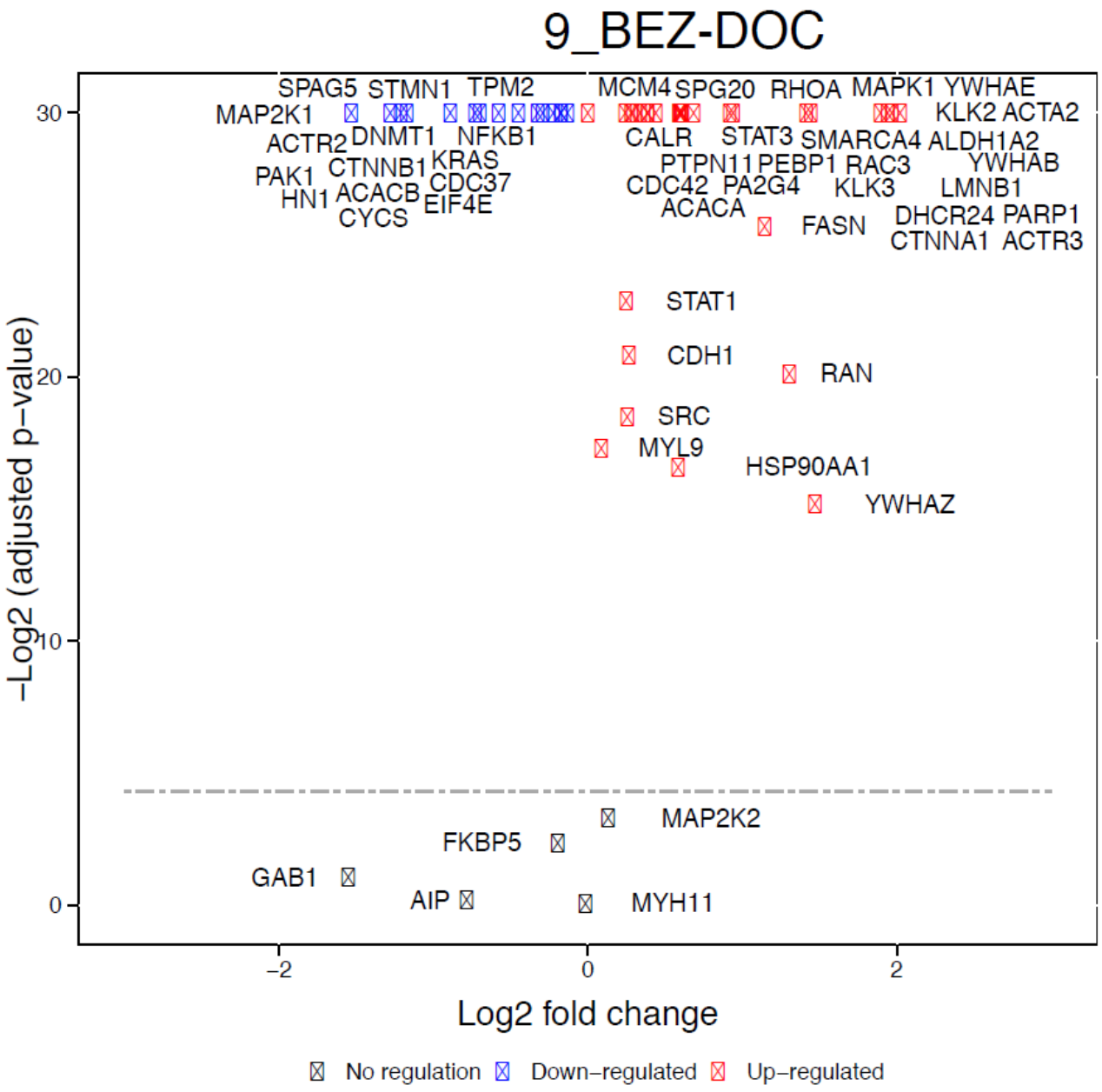
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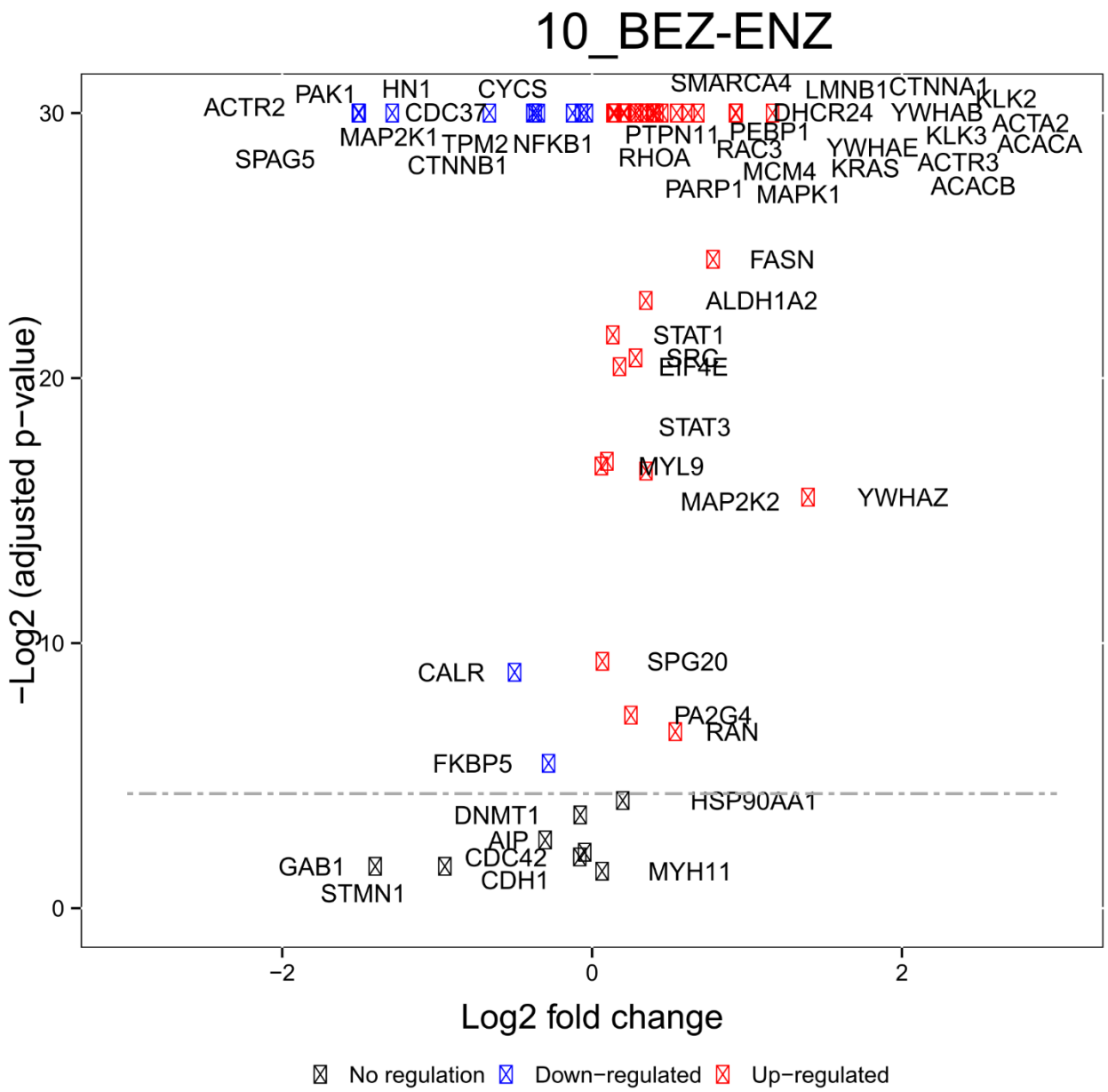
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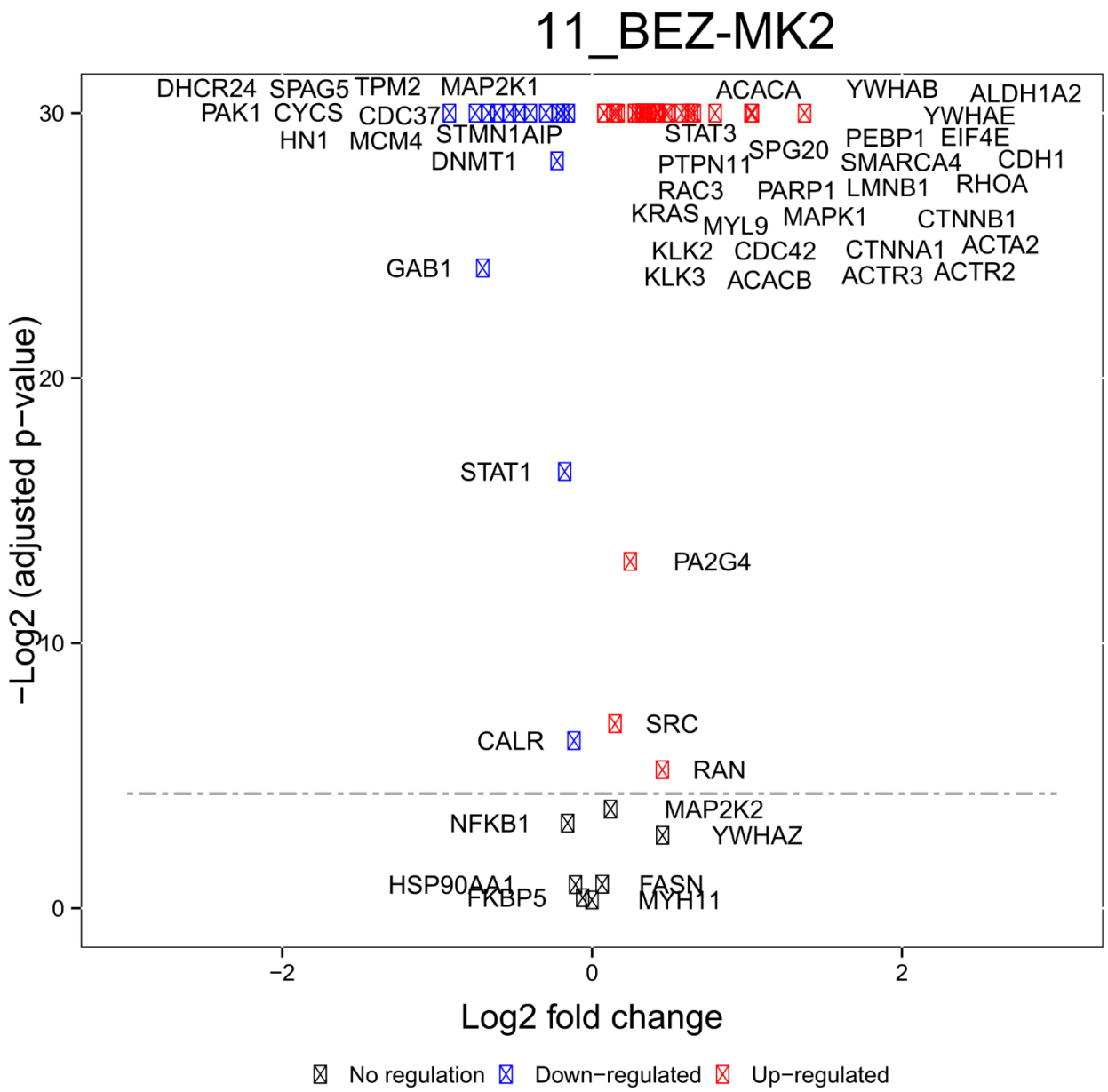
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STAT3
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RAN
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2
Log2 fold change
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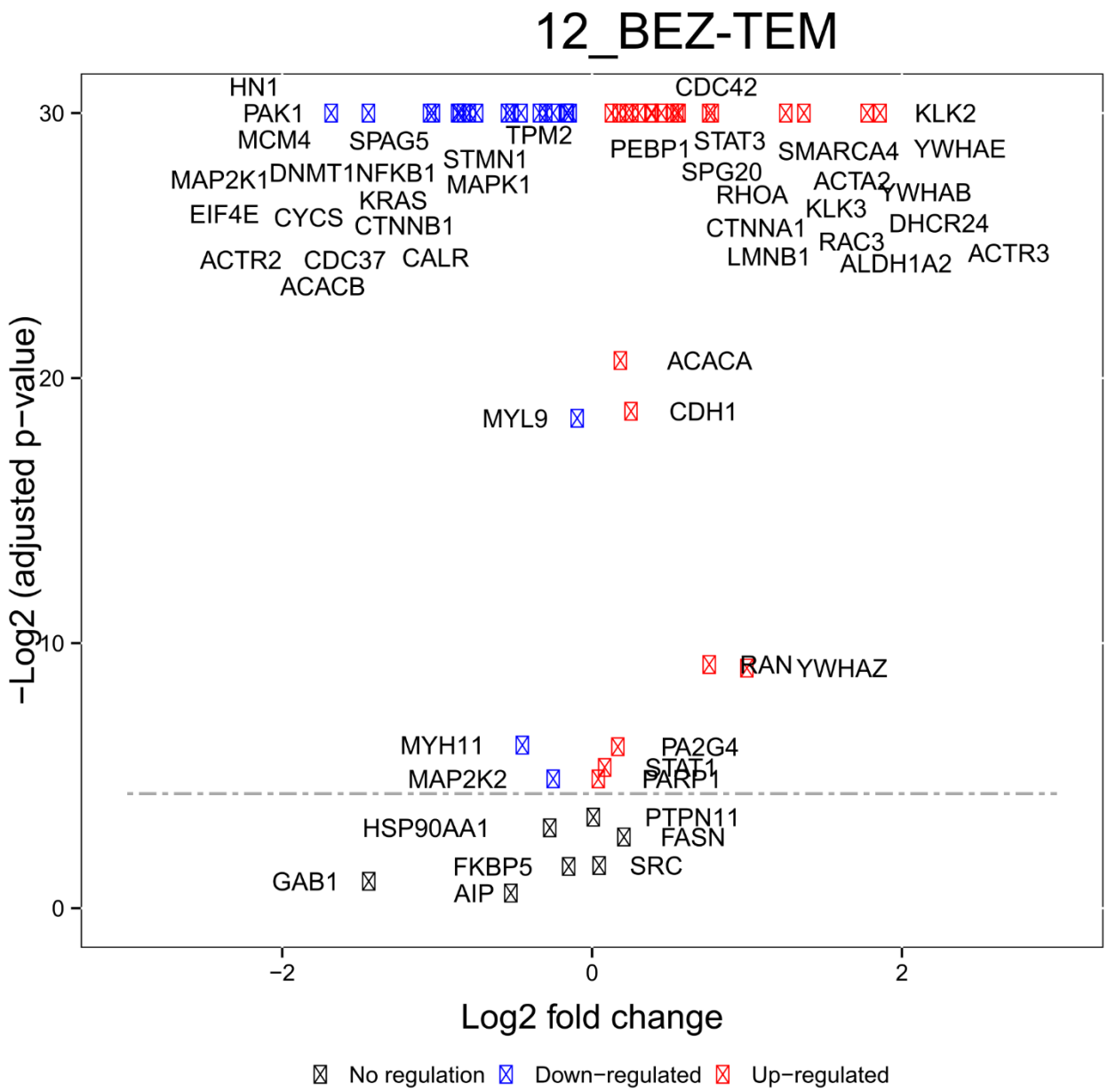
Up−regulated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ACACA
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Log2 fold change
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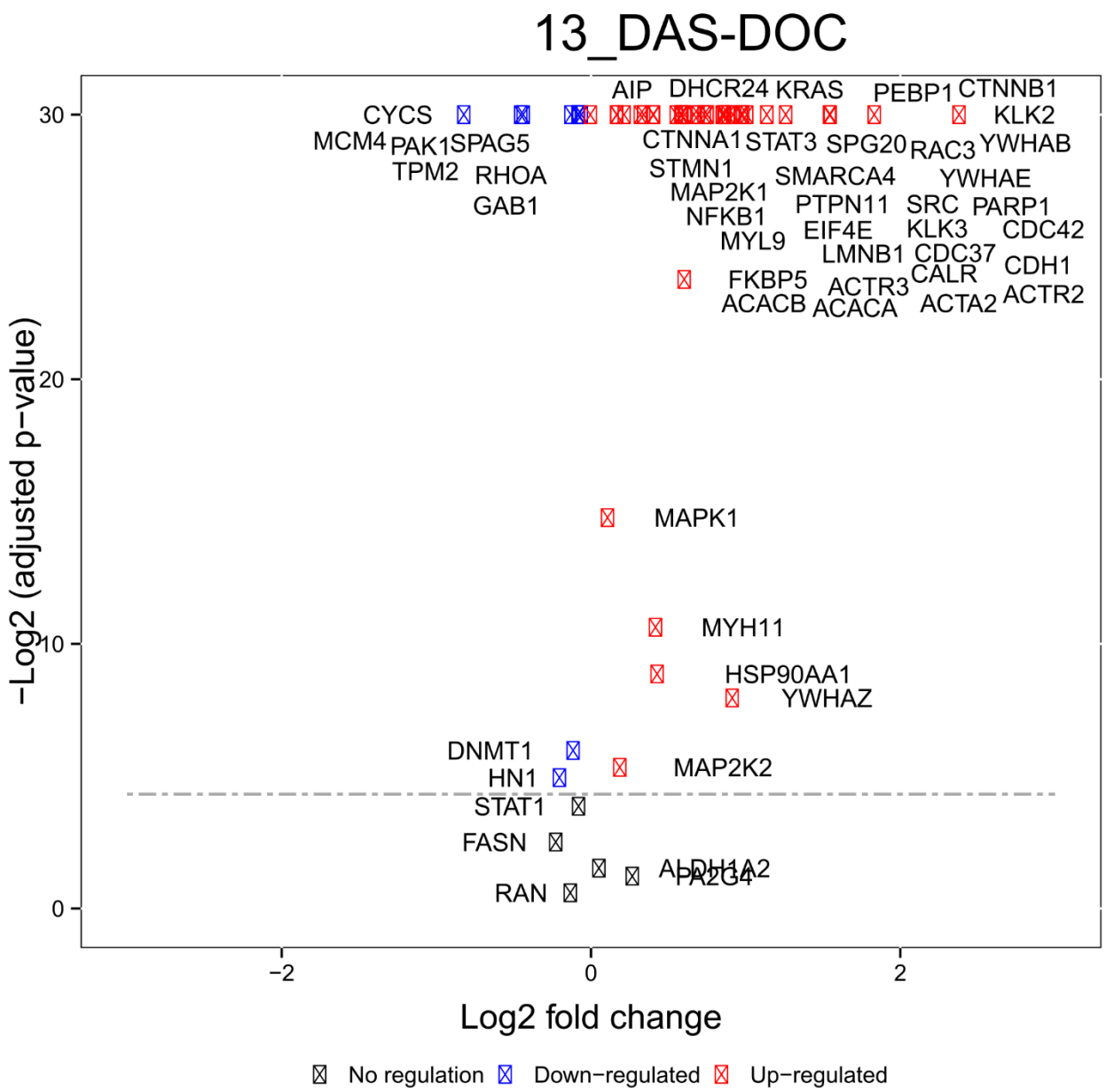
Up−regulated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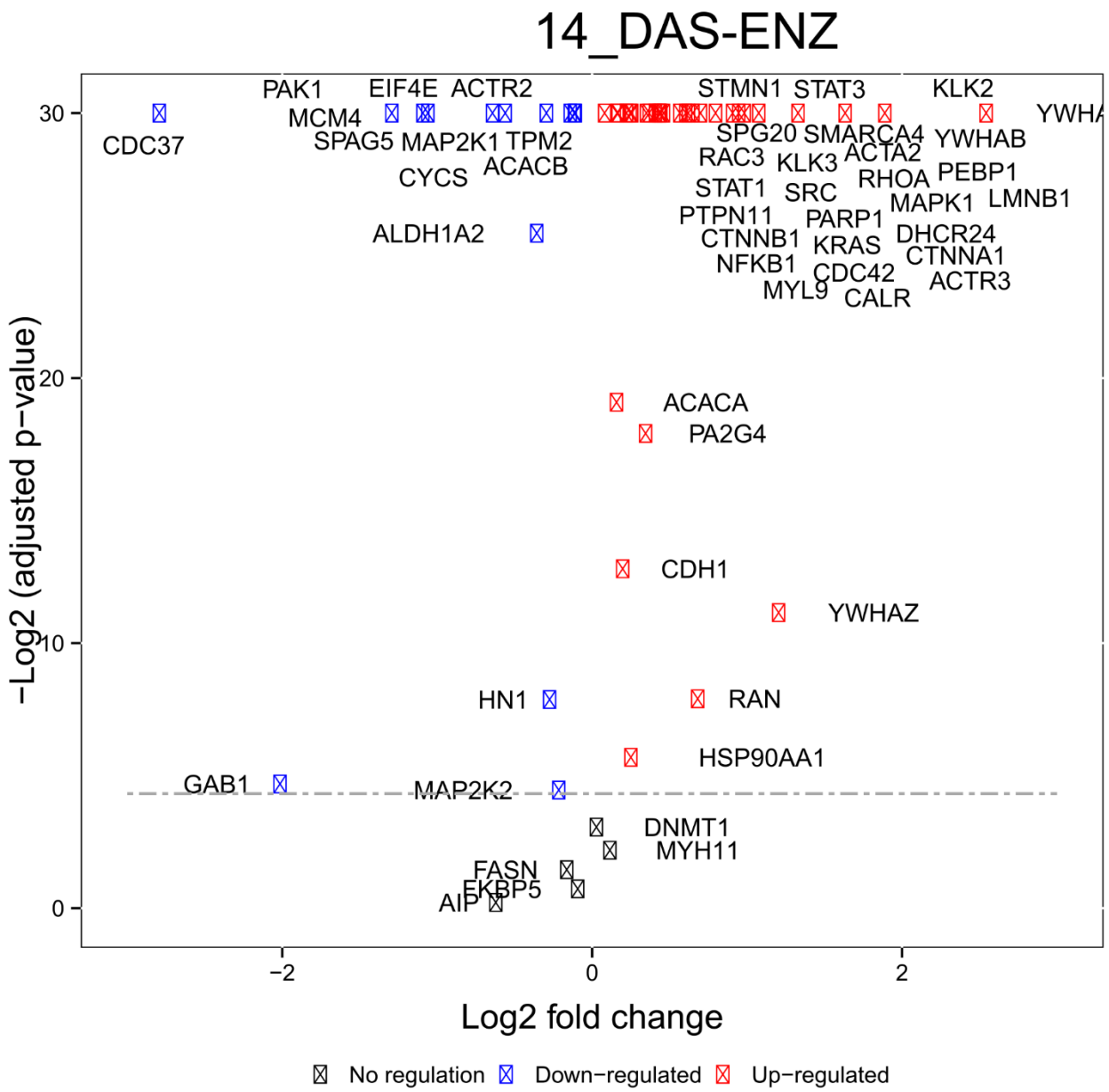
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RAC3
KLK3
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PEBP1
MAPK1
LMNB1
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PA2G4
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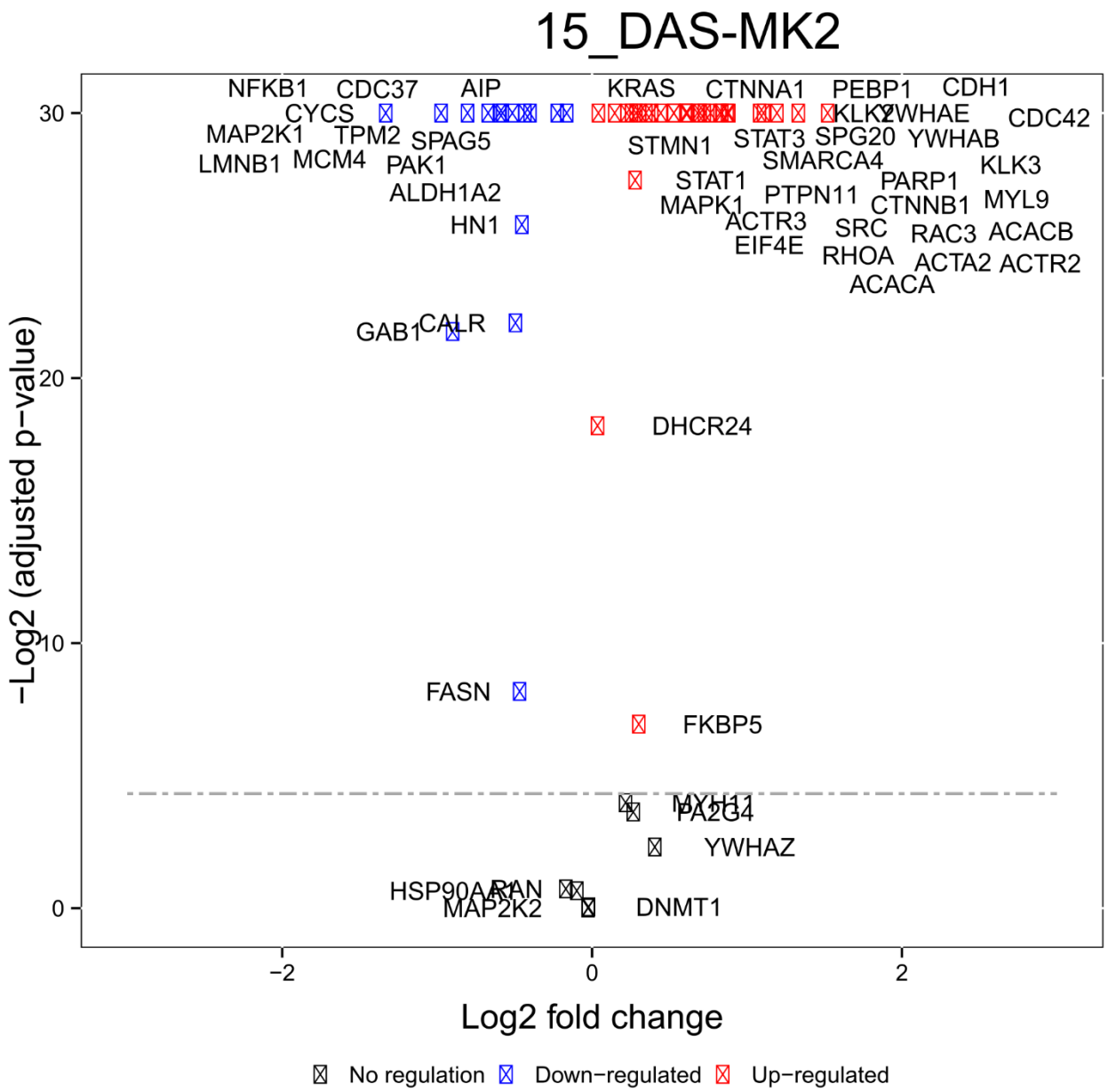
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HSP90AA1
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MAP2K2
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Log2 fold change
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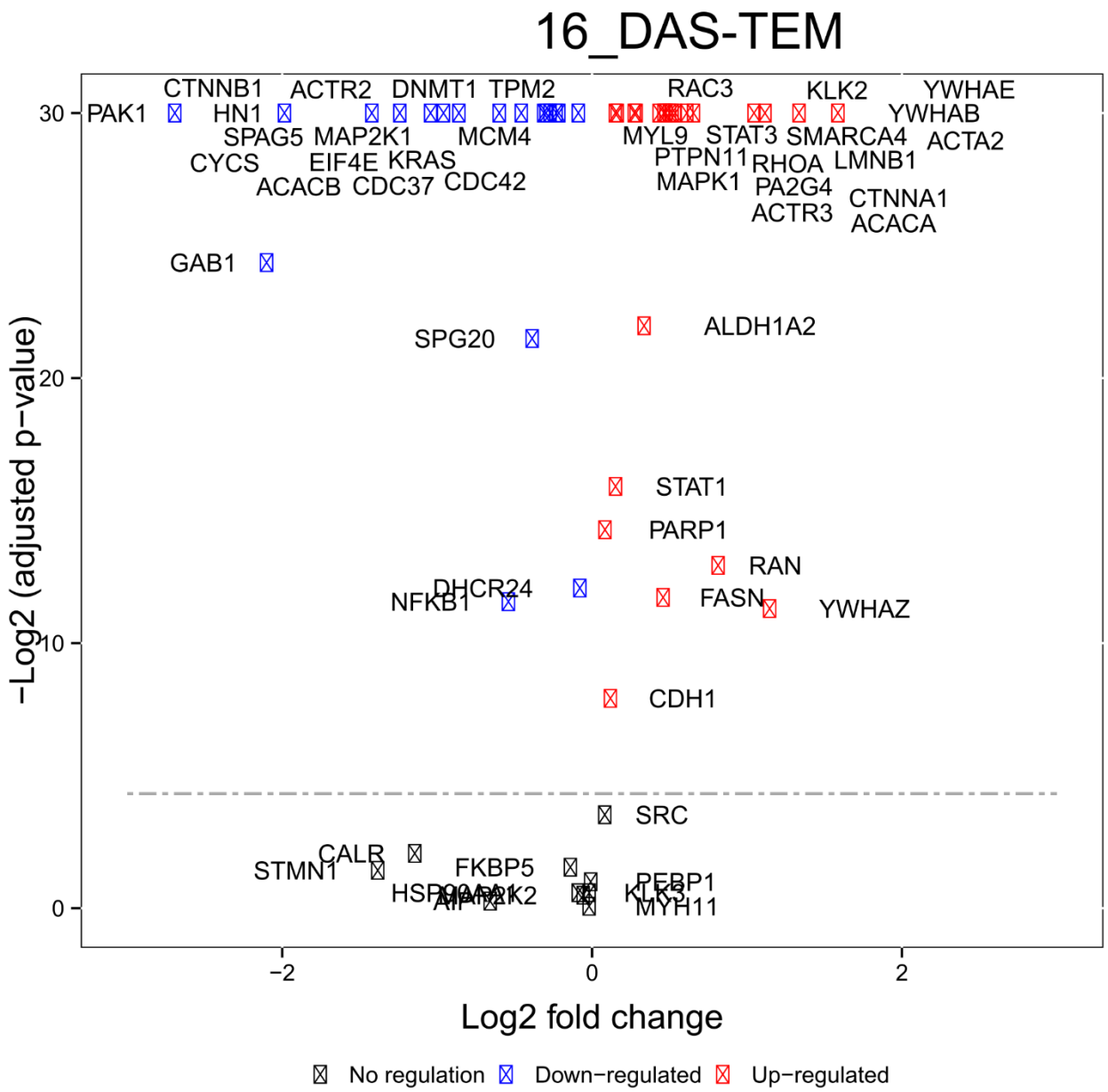
Up−regulated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MAPK1
PA2G4
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Log2 fold change
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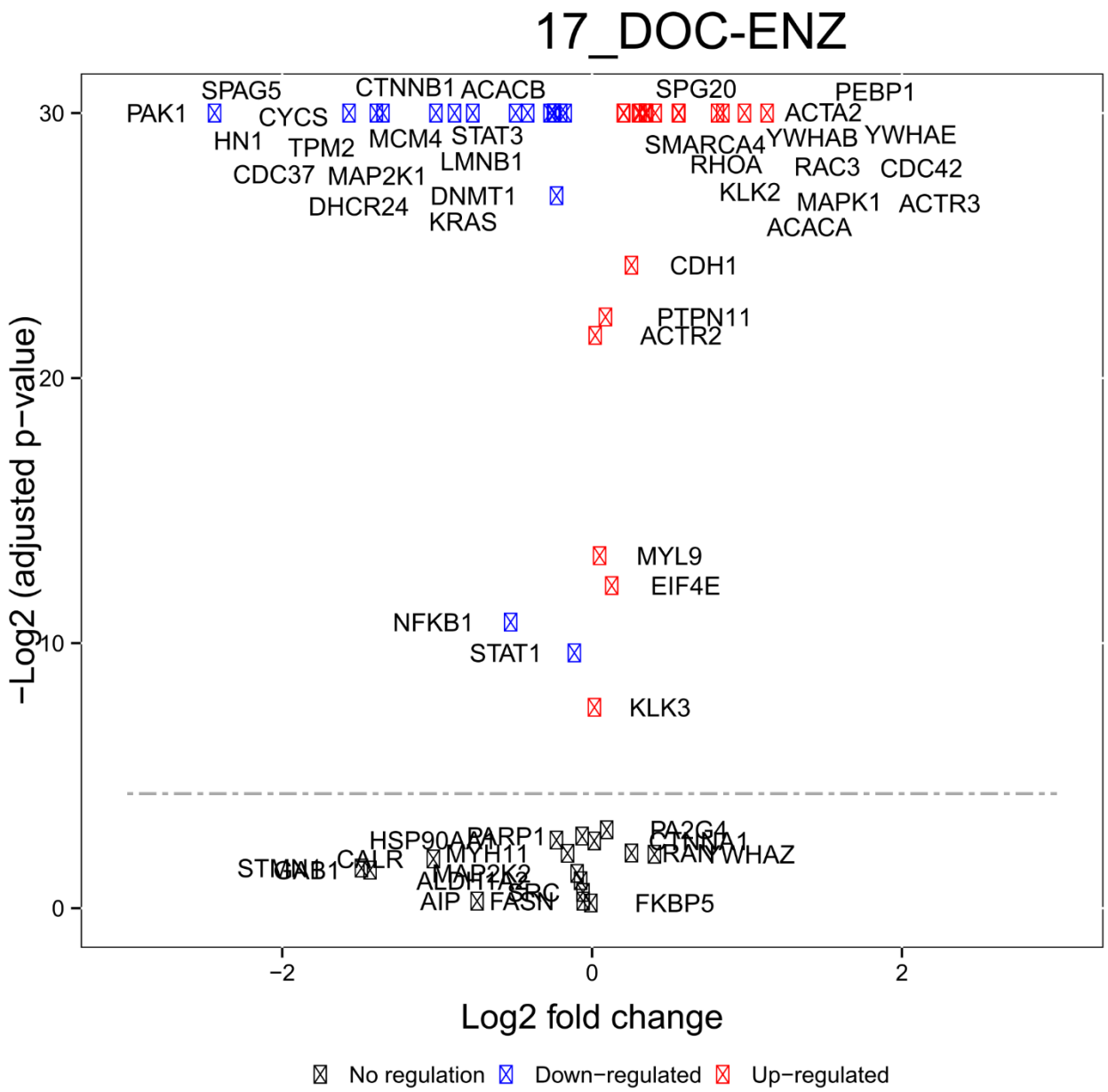
Up−regulated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YWHAE
LMNB1
RHOA
RAC3
CDC42
CDC37
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KLK2
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MAPK1
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PA2G4
CTNNA1
CALR
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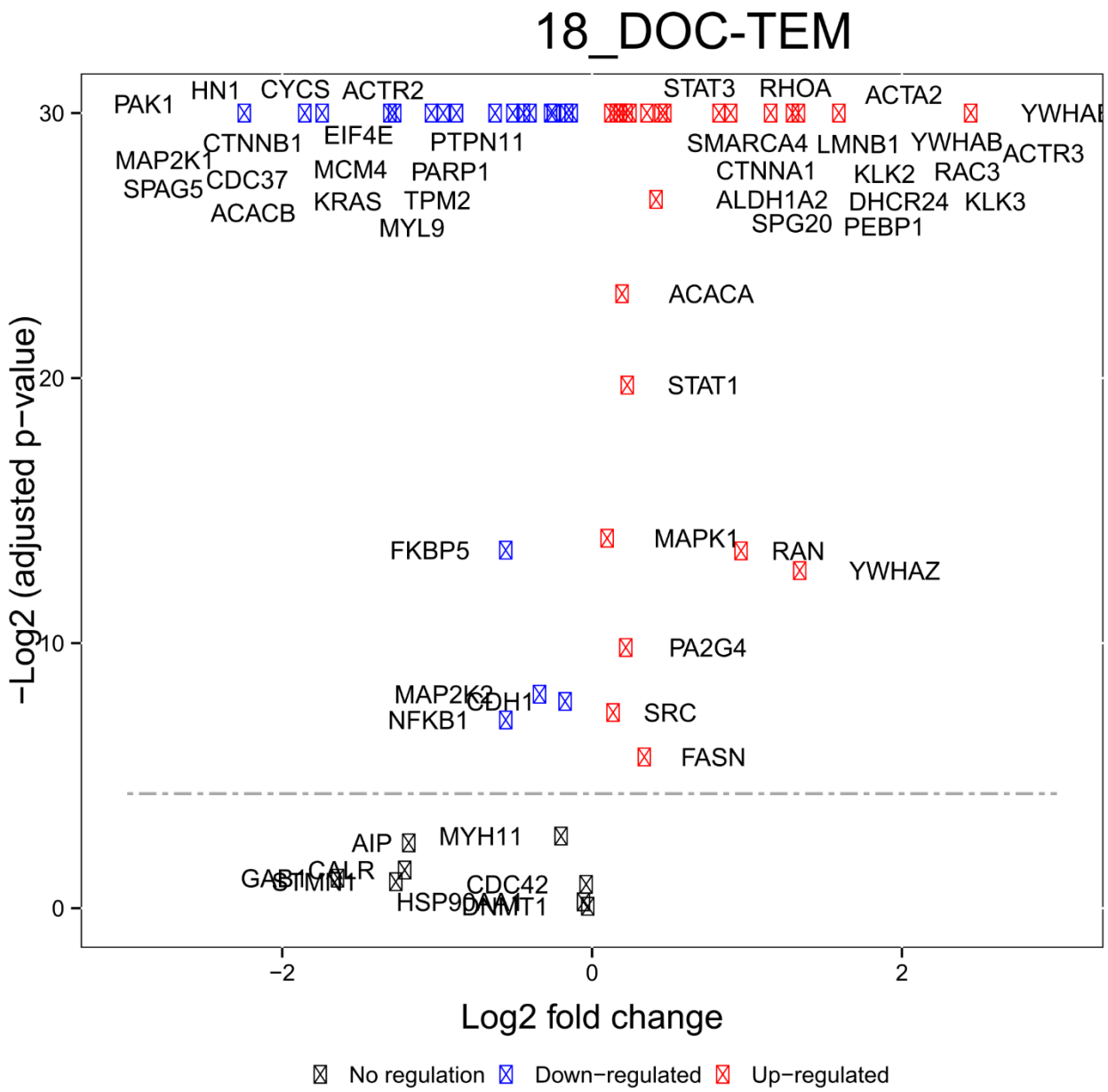
−Log2 (adjusted p−value)
Log2 fold change
No regulation
Down−regulated
Up−regulated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EIF4E
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YWHAB
ACTR3
MAP2K1
SPAG5
CDC37
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ACACB
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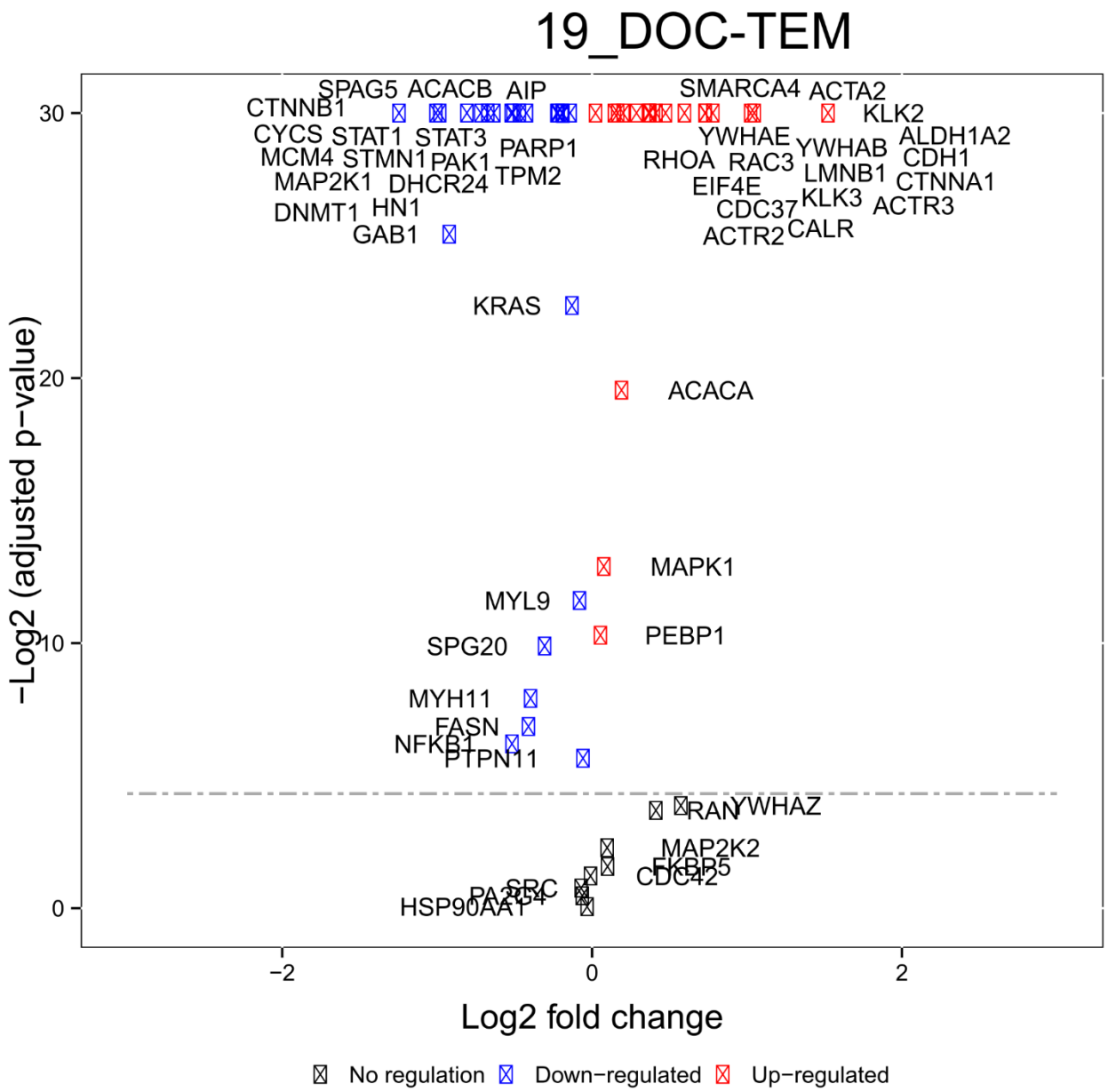
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STMN1
PAK1
RHOA
RAC3
YWHAB
CDH1
MAP2K1
DHCR24
TPM2
LMNB1
CTNNA1
EIF4E
DNMT1
HN1
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CDC37
GAB1
CALR
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MAP2K2
FKBP5
CDC42
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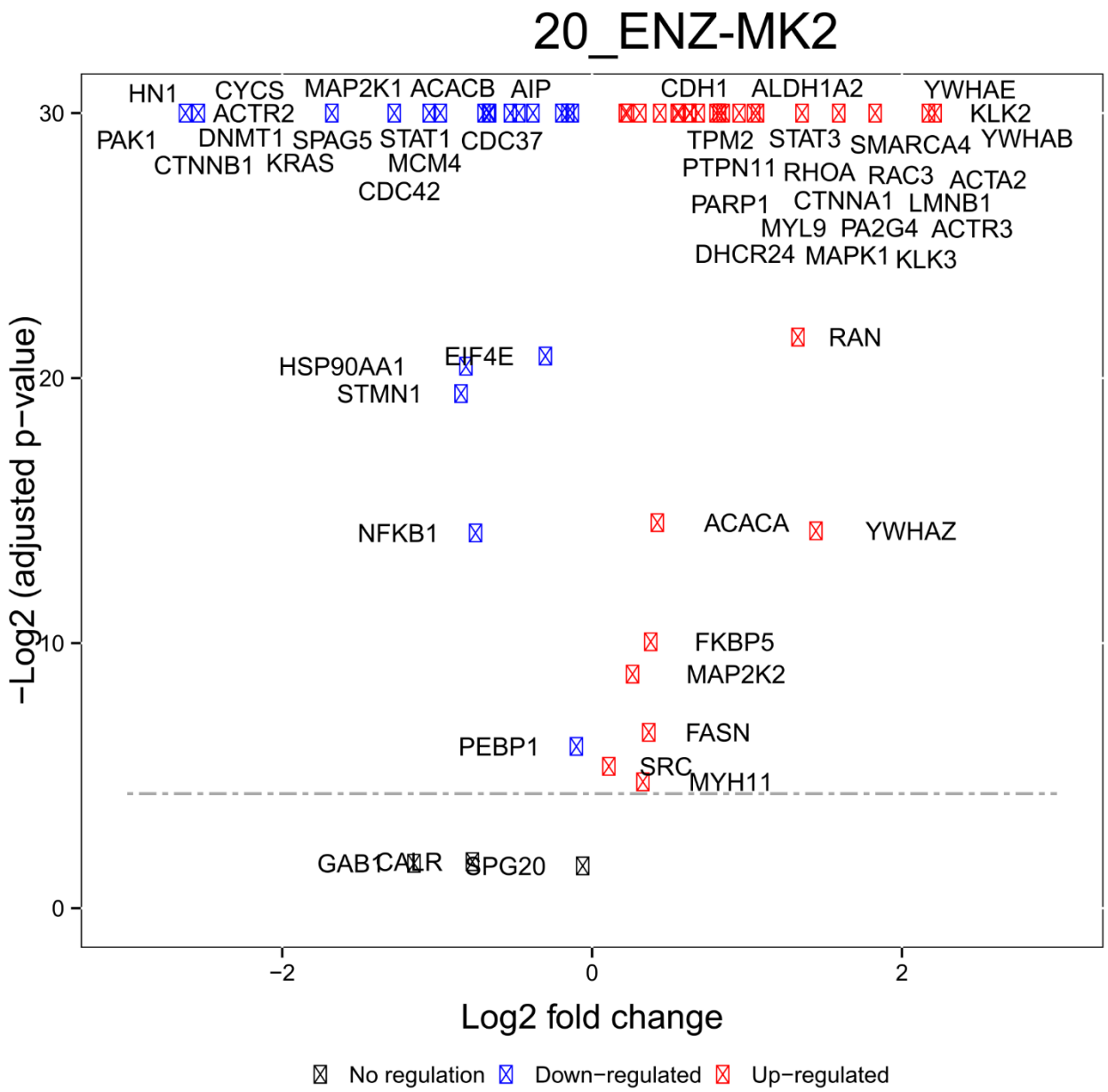
−2
0
2
Log2 fold change
No regulation
Down−regulated
Up−regulated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STAT1
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SMARCA4
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CTNNB1
KRAS
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PTPN11
RHOA
RAC3
ACTA2
CDC42
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PEBP1
SRC
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GAB1
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20
10
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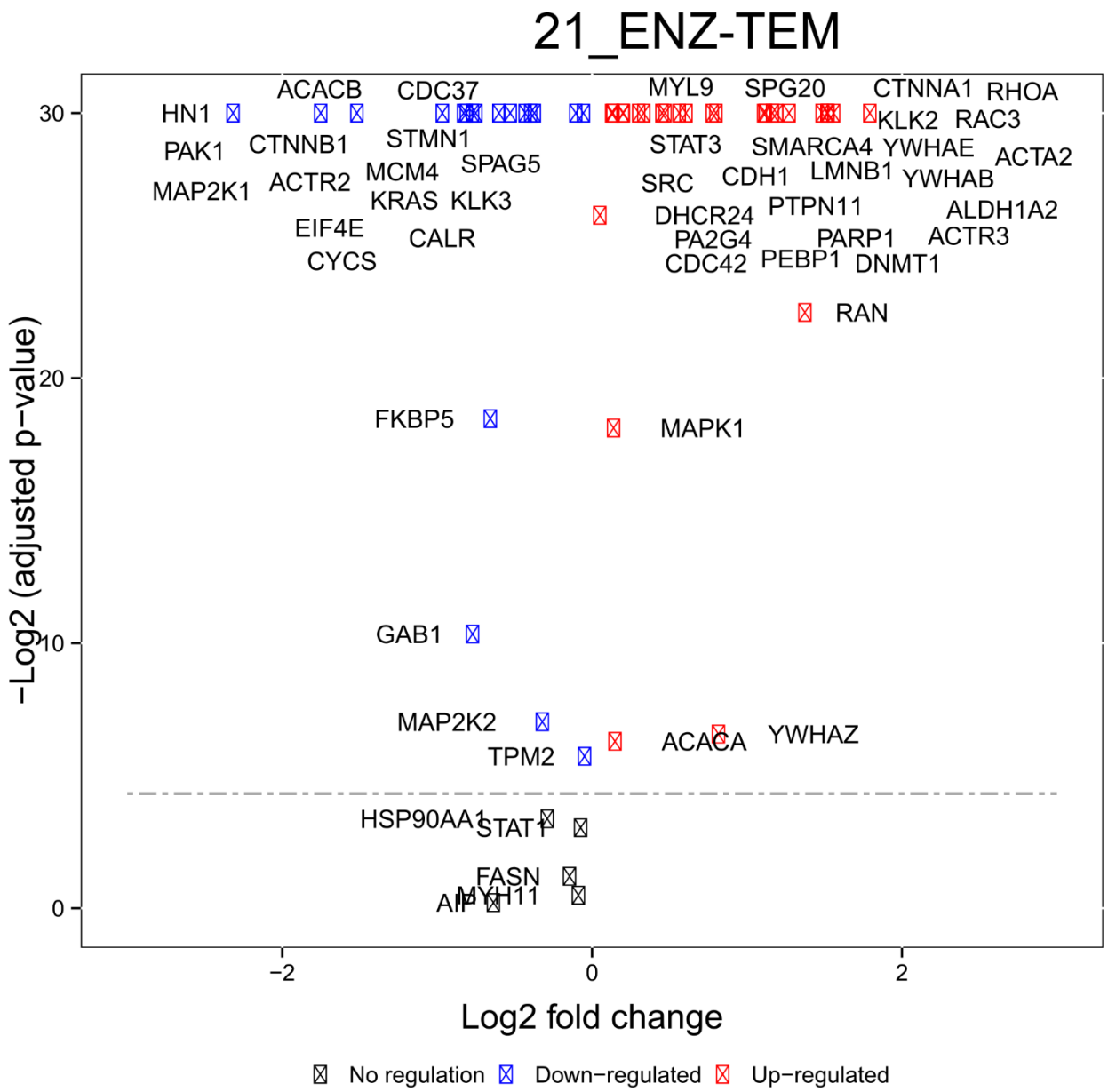
−2
0
2
Log2 fold change
No regulation
Down−regulated
Up−regulated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10
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−2
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Log2 fold change
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ACTA2
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MAP2K1
ACTR2
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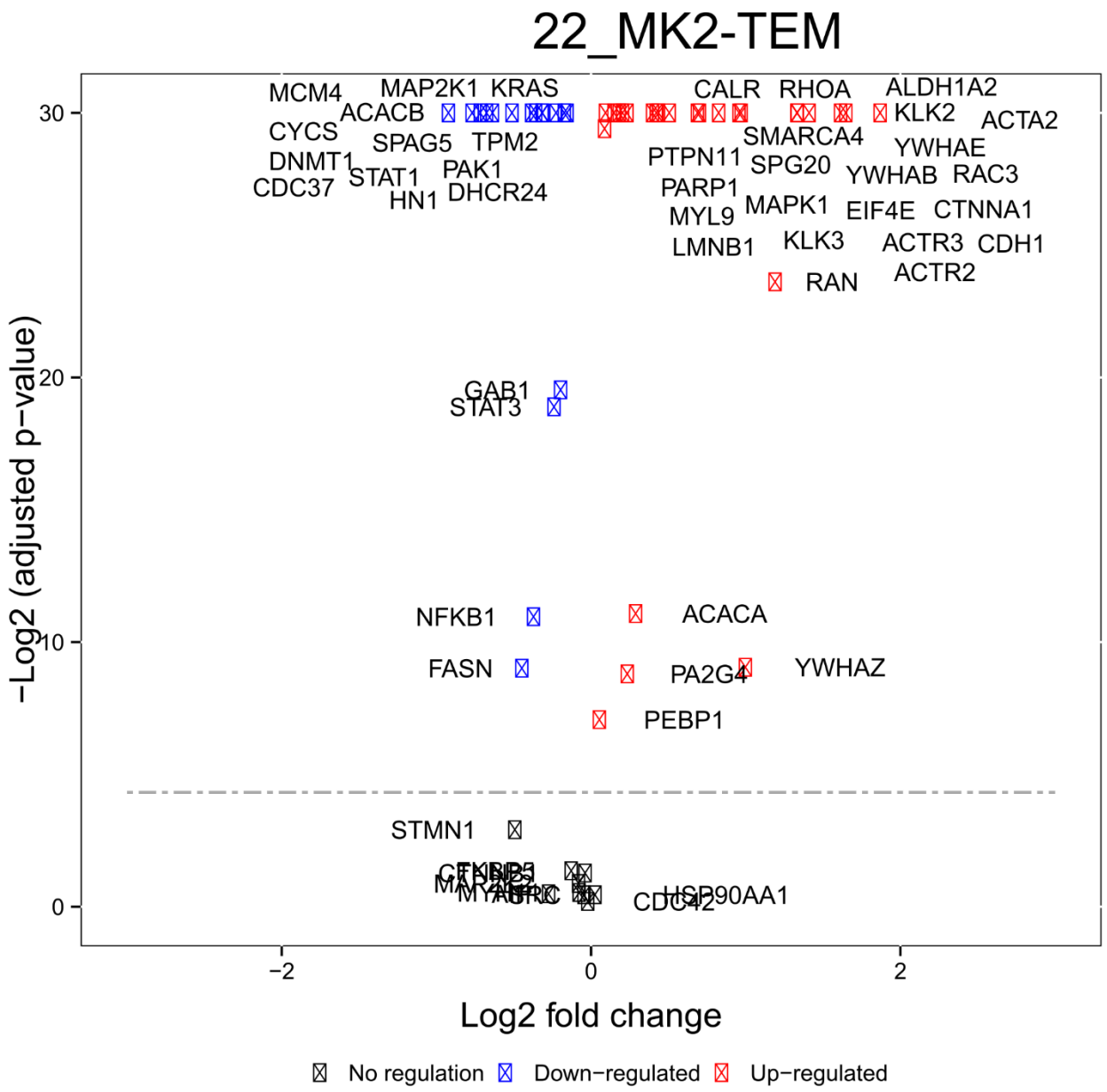
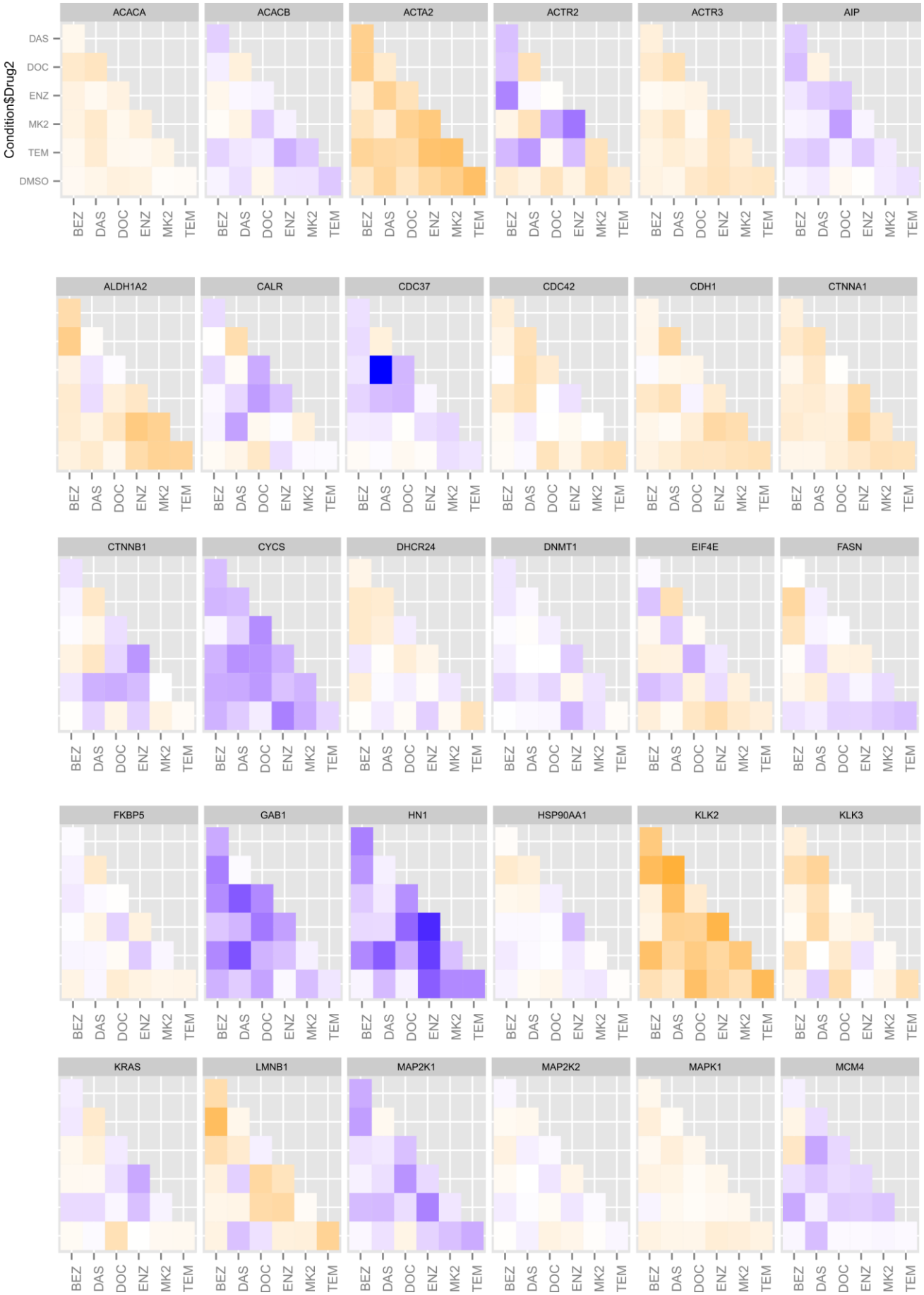
Up−regulated



Supplementary Figure 3: SRM data analysis I. 21 primary output of MSStats per condition plotting statistical significance as function of protein abundance ratio between treated and untreated.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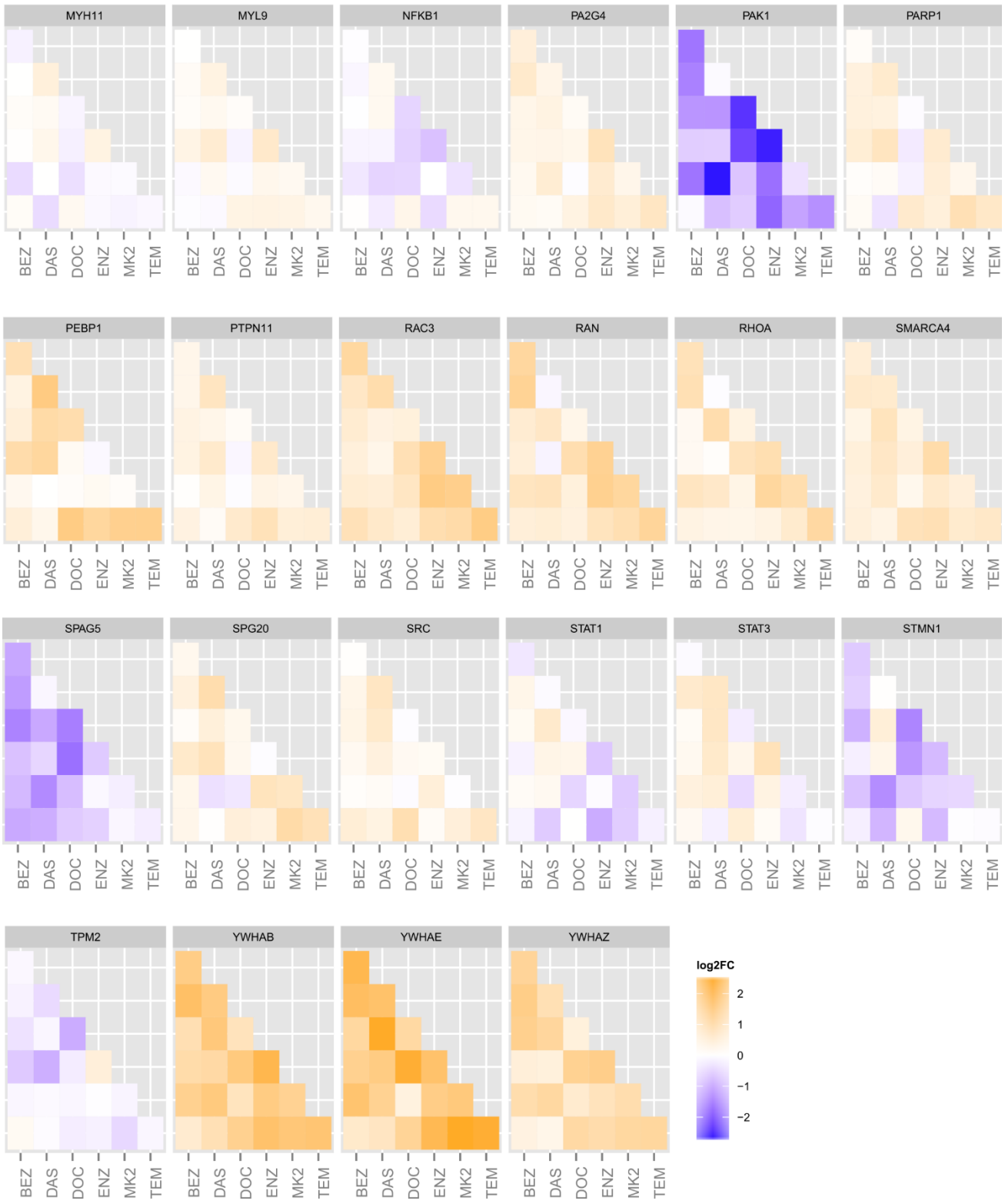
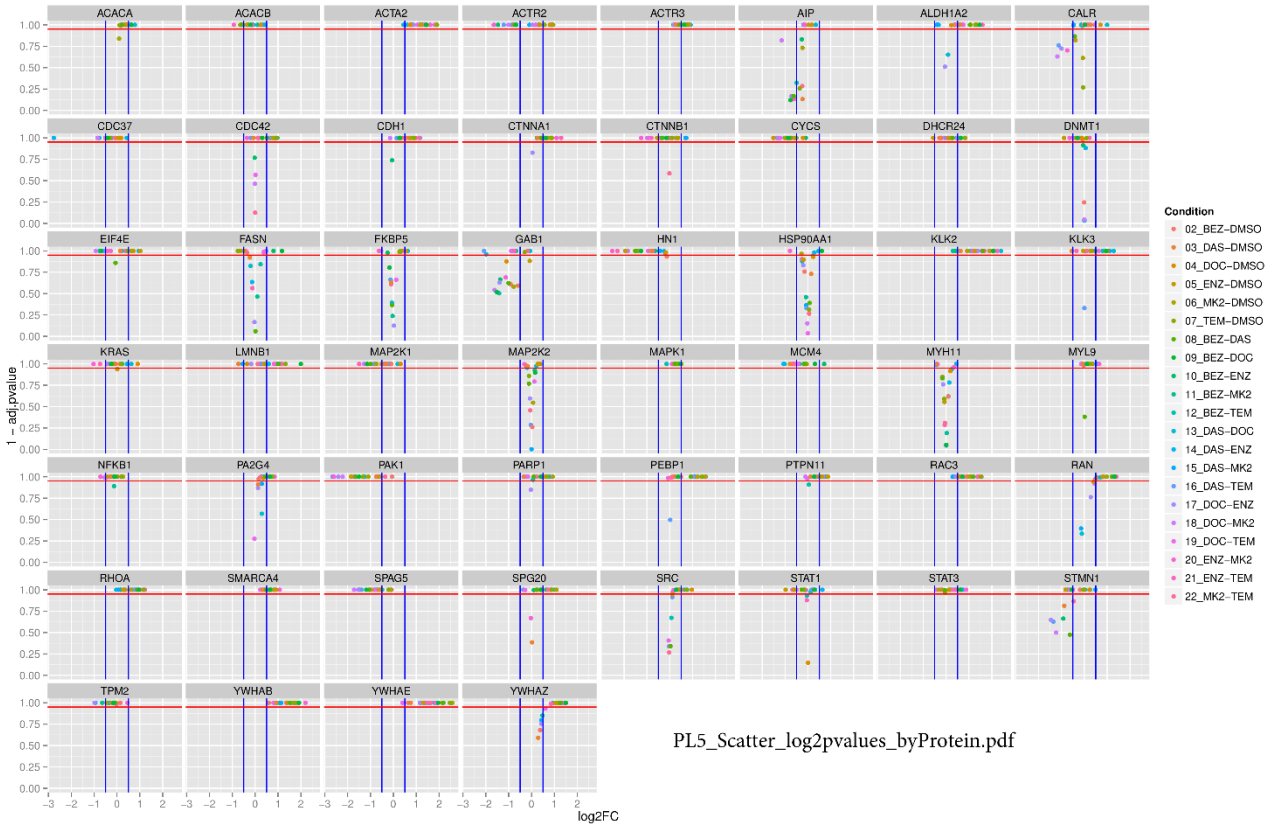


Supplementary Figure 4: SRM data analysis II. Protein centric abundance changes per condition relative to untreated. In these triangle plots the first drug is on the x-axis, while the second drug is along the y-axis. Protein names are official gene names.



Supplementary Figure 5: SRM data analysis III. A protein centric analysis plotting statistical significance as function of ratio between treatment condition to untreated. Protein names are official gene names.

Cell line	sample name	Drug 1	Concentration drug1	Drug 2	Concentration drug 2	Time point
Lncap	01_DMSO	DMSO	NA	DMSO	NA	24hrs
Lncap	02_BEZ-DMSO	Bez235	500nM	DMSO	NA	24hrs
Lncap	03_DAS-DMSO	Dasatinib	100nM	DMSO	NA	24hrs
Lncap	04_DOC-DMSO	Docetaxel	10nM	DMSO	NA	24hrs
Lncap	05_ENZ-DMSO	Enzalutamide	10µM	DMSO	NA	24hrs
Lncap	06_MK2-DMSO	MK2206	1µM	DMSO	NA	24hrs
Lncap	07_TEM-DMSO	Temsirolimus	100nM	DMSO	NA	24hrs
Lncap	08_BEZ-DAS	Bez235	500nM	Dasatinib	100nM	24hrs
Lncap	09_BEZ-DOC	Bez235	500nM	Docetaxel	10nM	24hrs
Lncap	10_BEZ-ENZ	Bez235	500nM	Enzalutamide	10µM	24hrs
Lncap	11_BEZ-MK2	Bez235	500nM	MK2206	1µM	24hrs
Lncap	12_BEZ-TEM	Bez235	500nM	Temsirolimus	100nM	24hrs
Lncap	13_DAS-DOC	Dasatinib	100nM	Docetaxel	10nM	24hrs
Lncap	14_DAS-ENZ	Dasatinib	100nM	Enzalutamide	10µM	24hrs
Lncap	15_DAS-MK2	Dasatinib	100nM	MK2206	1µM	24hrs
Lncap	16_DAS-TEM	Dasatinib	100nM	Temsirolimus	100nM	24hrs
Lncap	17_DOC-ENZ	Docetaxel	10nM	Enzalutamide	10µM	24hrs
Lncap	18_DOC-MK2	Docetaxel	10nM	MK2206	1µM	24hrs
Lncap	19_DOC-TEM	Docetaxel	10nM	Temsirolimus	100nM	24hrs
Lncap	20_ENZ-MK2	Enzalutamide	10µM	MK2206	1µM	24hrs
Lncap	21_ENZ-TEM	Enzalutamide	10µM	Temsirolimus	100nM	24hrs
Lncap	22_MK2-TEM	MK2206	1µM	Temsirolimus	100nM	24hrs

Supplementary MM 1: Perturbation matrix. List of 21 treatment conditions plus vehicle (DMSO) control with drug concentrations.

Cell Lysis: Reagents	**Procedure**			
Lysis buffer	resuspend cell pellets in 100 µL **Lysis buffer** (up/down pipetting)			
71296 | PhosphoSafe™ Extraction Reagent from Millipore	sonicate 1.5 mL tubes with 90% intensity 0.8 cycle time (on/off) 20x			
	BCA assay			
Reduction and Alkylation: Reagents	**Reduction and Alkylation: Procedure**			
0.2 M TCEP in 0.1 M HEPES (stored @ -20°C)	Add 4 µL of 0.2 M TCEP			☐
Tris(2-carboxyethyl)phosphine hydrochloride	Incubate for 15 min @ 37 °C on a shaker with 1,400 rpm			
MW 286.65 g/mol = 29 mg in 500 µL	Cool down the sample to room temperature			
	Add 4 µL of 0.4 M iodoacetamide			☐
0.4 M iodoacetamide in 0.1 M HEPES	Incubate for 30 min @ 25 °C on a shaker with 1,400 rpm in the dark			
MW 184.96 g/mol = 37 mg in 500 µL	Spin down 5 min at 12,000 rcf @ 20 °C			
Trypsin digest: Reagents	**Trypsin digest: Procedure**			
Trypsin, sequencing grade modified	Add 3 µL 1 M NaOH, Check pH			☐
V511C, Promega; stored @ -80 °C	add trypsin (2 µL or 1:100 dil.)			
aliquots of 20 µg (40 µL, 0.5 µg/µL)	incubate overnight at 37 °C and 250 rpm (floor shaker)			
SDS removal: Reagents	**SDS removal: Procedure**			
Detergent Removal Resin, Columns	remove bottom plug (top only after first spin, resin inside!)			
(Thermo Scientific, 877777)	centrifuge for 1 min @ 1'500 rcf			
SAMPLE VOLUME 25 - 250 µL	add 0.4 mL 0.1 M HEPES		3x	☐
	place in new collection tube, add 25 -250 µL sample (1:1 ratio)			
	close lid, vortex, incubate for 10 min at room temp			
	centrifuge for 2 min @ 1'500 rcf			
	collect SDS free peptides			
C18 cleanup: Reagents	**C18 cleanup: Procedure**			
Sep-Pak Vac 1cc (50 mg) WAT036820 tC18 Cartridges	Adjust pH to 2-3 using 10 % TFA (approx 8 µL)			☐
Wash C18	apply supernatante to C18			
5 % acetonitrile	1 mL acetonitrile	VAC		
0.1 % trifluoroacetic acid (TFA)	1 mL **Wash C18**	VAC	3x	☐
	Apply sample	vac OFF		
Eulte C18	1 mL **Wash C18**	VAC	3x	☐
50 % acetonitrile	0.5 mL **Elute C18**	syringe		
0.1 % trifluoroacetic acid (TFA)	DRY under vaccum 45 °C			
HILIC	**HILIC**			
Harvard Apparatus GmbH	add 400 µL 100 % methanol			
Macro SpinColumns, Hydrophilic Packing Material	centrifuge for 2 min @ 1100 rcf			
Qty. of 96; 744305	discard flowthrough			
	add 400 µL **dH_2O**			
Conditioning Buffer pH 4	centrifuge for 1 min @ 1100 rcf		3x	☐
0.2 M monosodium acetate,	discard flowthrough			
0.3 M sodium acetate	add 600 µL **Conditioning Buffer pH 4**			
	centrifuge for 30 sec. @ 1100 rcf			
Loading Buffer pH 6	**let equilibrate for 1 hour**			
25 mM ammonium formate	centrifuge for 1 min @ 1100 rcf			
80 % acetonitrile	add 400 µL **Loading Buffer pH 6**			
	centrifuge for 1 min @ 1100 rcf		3x	☐
	discard flowthrough			
Elute HILIC	resuspend dried peptides in 400 µL **Loading Buffer pH 6**			
2 % formic acid, HPLC water	sonicate sample, 5 min			
	centrifuge for 1 min @ 1100 rcf			
	discard flowthrough, add HILIC columns to a new 2 mL tube			
	add 400 µL **Elute HILIC**			
	centrifuge for 2 min @ 1100 rcf			
	dry under vaccum 45 °C			

Supplementary MM 2: Peptide generation protocol. Step-by-step protocol, including reagents used, to generate purified peptides free of neutral lipids.