Electronic Supplementary Material (ESI) for Molecular BioSystems

qPCA: scalable assay to study the perturbation of protein-protein interactions in living cells

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Supplementary information

Supplementary Tables

Table S1. Protein-protein interactions selected to test for the relationship between growth and the amount of DHFR complex formed.

Gene A-DHFR F[1,2]	Gene B-DHFR F[3]	Interactions (Gene names)	Interaction (ORF names)	Abundance
VMA21	VPH1	VMA21-VPH1	YGR105W-YOR270C	H
ARX1	YBR267W	ARX1-YBR267W	YDR101C-YBR267W	H
DHH1	EDC3	DHH1-EDC3	YDL160C-YEL015W	H
DHH1	LSM7	DHH1-LSM7	YDL160C-YNL147W	H
DHH1	PBP1	DHH1-PBP1	YDL160C-YGR178C	H
SGN1	PUB1	SGN1-PUB1	YIR001C-YNL016W	H
TOM70	ALO1	TOM70-ALO1	YNL121C-YML086C	H
CKB1	CKA2	CKB1-CKA2	YGL019W-YOR061W	M
NOT5	MOT2	NOT5-MOT2	YPR072W-YER068W	M
LSB3	CUE5	LSB3-CUE5	YFR024C-A-YOR042W	L
MMS2	SIP5	MMS2-SIP5	YGL087C-YMR140W	L
PEX14	PEX17	PEX14-PEX17	YGL153W-YNL214W	L
SLA1	END3	SLA1-END3	YBL007C-YNL084C	L
YKE2	GIM5	YKE2-GIM5	YLR200W-YML094W	L
VPS29	VPS35	VPS29-VPS35	YHR012W-YJL154C	L

For each pair we used the data by *Ghaemmaghami et al.* [1] and we calculated the average abundance. Then, we classified the pairs in 3 classes: low abundance (L), medium abundance (M) and high abundance (H).

References

1. S. Ghaemmaghami, W. Huh, K. Bower, R. W. Howson, A. Belle, N. Dephoure, E. K. O'Shea and J. S. Weissman, *Nature*, 2003, **425**, 737-741.

Table S2. Genotypes of the strains constructed in this study.

Strain	Genotype
LTQ001	*MATa, VMA21-DHFR F[1,2]-natNT2, VPH1-DHFR F[3]-hphNT1*
LTQ002	*MATa, ARX1-DHFR F[1,2]-natNT2, YBR267W-DHFR F[3]-hphNT1*
LTQ003	*MATa, DHH1-DHFR F[1,2]-natNT2, EDC3-DHFR F[3]-hphNT1*
LTQ004	*MATa, DHH1-DHFR F[1,2]-natNT2, LSM7-DHFR F[3]-hphNT1*
LTQ005	*MATa, DHH1-DHFR F[1,2]-natNT2, PBP1-DHFR F[3]-hphNT1*
LTQ006	*MATa, SGN1-DHFR F[1,2]-natNT2, PUB1-DHFR F[3]-hphNT1*
LTQ007	*MATa, TOM70-DHFR F[1,2]-natNT2, ALO1-DHFR F[3]-hphNT1*
LTQ008	*MATa, CKB1-DHFR F[1,2]-natNT2, CKA2-DHFR F[3]-hphNT1*
LTQ009	*MATa, NOT5-DHFR F[1,2]-natNT2, MOT2-DHFR F[3]-hphNT1*
LTQ010	*MATa, LSB3-DHFR F[1,2]-natNT2, CUE5-DHFR F[3]-hphNT1*
LTQ011	*MATa, MMS2-DHFR F[1,2]-natNT2, SIP5-DHFR F[3]-hphNT1*
LTQ012	*MATa, PEX14-DHFR F[1,2]-natNT2, PEX17-DHFR F[3]-hphNT1*
LTQ013	*MATa, SLA1-DHFR F[1,2]-natNT2, END3-DHFR F[3]-hphNT1*
LTQ014	*MATa, YKE3-DHFR F[1,2]-natNT2, GIM5-DHFR F[3]-hphNT1*
LTQ015	*MATa, VPS29-DHFR F[1,2]-natNT2, VPS35-DHFR F[3]-hphNT1*
LTQ016	*MAT α, VMA21-DHFR F[1,2]-natNT2, VPH1-DHFR F[3]-hphNT1*
LTQ017	*MAT α, ARX1-DHFR F[1,2]-natNT2, YBR267W-DHFR F[3]-hphNT1*
LTQ018	*MAT α, DHH1-DHFR F[1,2]-natNT2, EDC3-DHFR F[3]-hphNT1*
LTQ019	*MAT α, DHH1-DHFR F[1,2]-natNT2, LSM7-DHFR F[3]-hphNT1*
LTQ020	*MAT α, DHH1-DHFR F[1,2]-natNT2, PBP1-DHFR F[3]-hphNT1*
LTQ021	*MAT α, SGN1-DHFR F[1,2]-natNT2, PUB1-DHFR F[3]-hphNT1*
LTQ022	*MAT α, TOM70-DHFR F[1,2]-natNT2, ALO1-DHFR F[3]-hphNT1*
LTQ023	*MAT α, CKB1-DHFR F[1,2]-natNT2, CKA2-DHFR F[3]-hphNT1*
LTQ024	*MAT α, NOT5-DHFR F[1,2]-natNT2, MOT2-DHFR F[3]-hphNT1*
LTQ025	*MAT α, LSB3-DHFR F[1,2]-natNT2, CUE5-DHFR F[3]-hphNT1*
LTQ026	*MAT α, MMS2-DHFR F[1,2]-natNT2, SIP5-DHFR F[3]-hphNT1*

LTQ027	*MAT α, PEX14-DHFR F[1,2]-natNT2, PEX17-DHFR F[3]-hphNT1*
LTQ028	*MAT α, SLA1-DHFR F[1,2]-natNT2, END3-DHFR F[3]-hphNT1*
LTQ029	*MAT α, YKE3-DHFR F[1,2]-natNT2, GIM5-DHFR F[3]-hphNT1*
LTQ030	*MAT α, VPS29-DHFR F[1,2]-natNT2, VPS35-DHFR F[3]-hphNT1*
JFL001	*MATa/MAT α, TPK1-DHFR F[1,2]-natNT2/TPK1, BCY1/ BCY1-DHFR F[3]-hphNT1*
JFL002	*MATa/MAT α, TPK2-DHFR F[1,2]-natNT2/TPK2, BCY1/BCY1-DHFR F[3]-hphNT1*
JFL003	*MAT α, TPK1-DHFR F[1,2]-natNT2, BCY-DHFR F[3]-hphNT1*
JFL004	*MAT α, TPK2-DHFR F[1,2]-natNT2, BCY-DHFR F[3]-hphNT1*
JFL005	*MATa/MAT α, TPK1/TPK1-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, pde1Δ-KanMX/PDE1*
JFL006	*MATa/MAT α, TPK1/TPK1-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, pde2Δ-KanMX/PDE2*
JFL007	*MATa/MAT α, TPK2/TPK2-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, pde1Δ-KanMX/PDE1*
JFL008	*MATa/MAT α, TPK2/TPK2-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, pde2Δ-KanMX/PDE2*
JFL009	*MATa/MAT α,TPK1/ TPK1-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, hoΔ-KanMX/HO*
JFL010	*MATa/MAT α, TPK2/TPK2-DHFR F[1,2]-natNT2, BCY1/BCY1-DHFR F[3]-hphNT1, hoΔ-KanMX/HO*
JFL011	*MATa/MAT α, TPK2-Myc-hphNT1/TPK2, BCY1/BCY1-HA-natNT2*

Table S3. Oligonucleotides used in this study.

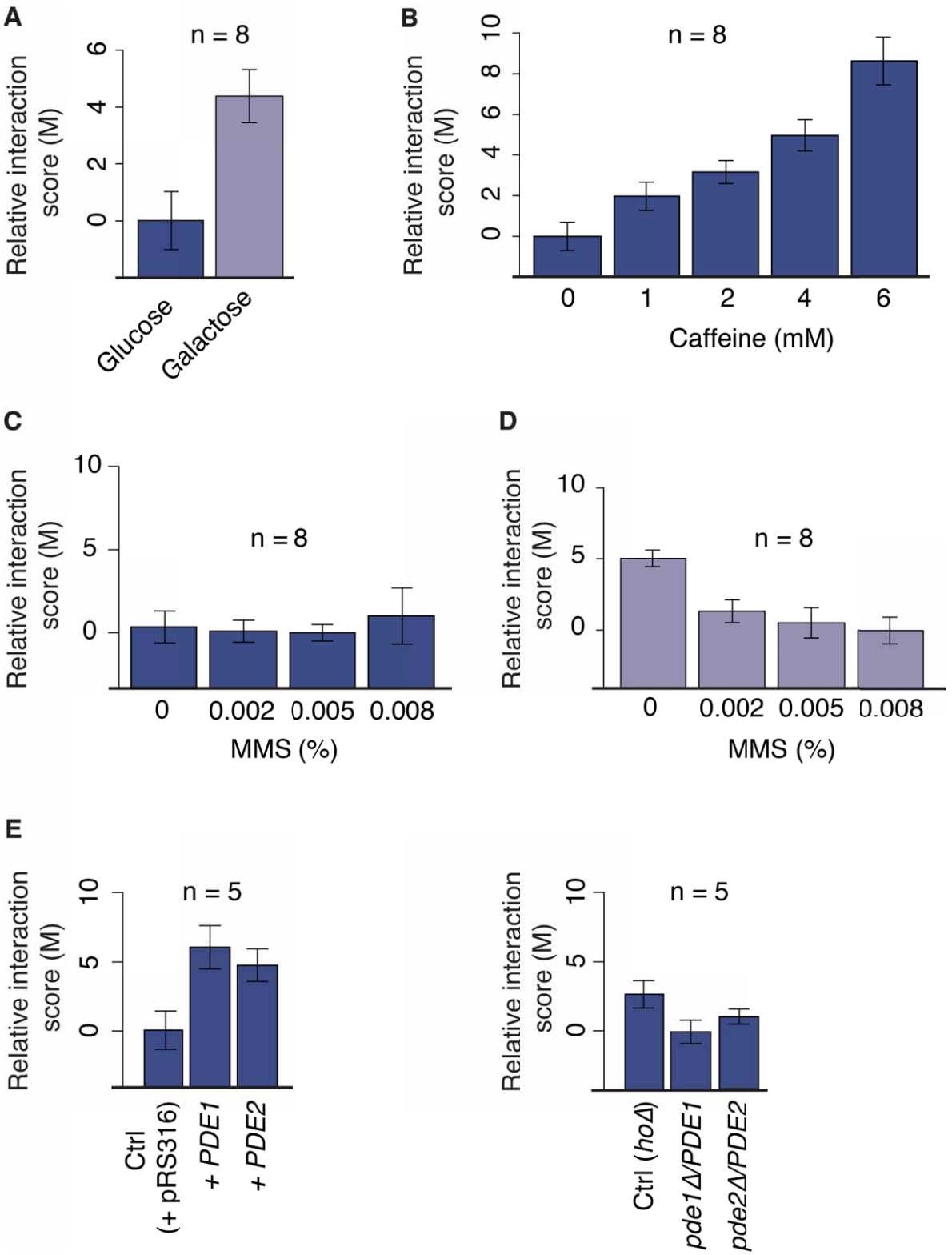
Experiments	Primer Information	Primer Sequence 5' to 3'
qPCA	C Oligo Forward YGR105W (VMA21)	GTTTAGCTGCTGCAATGGCC
qPCA	C Oligo Forward YOR270C (VPH1)	AAGTTTTTCGTGGGTGAAGG
qPCA	C Oligo Forward YDR101C (ARX1)	GCCAAGGATAAGAGGTTCGG
qPCA	C Oligo Forward YBR267W (YBR267W)	GACTCAACAGCGTGTTTGGC
qPCA	C Oligo Forward YDL160C (DHH1)	ACAGGCGTATCCTCCACCGC
qPCA	C Oligo Forward YEL015W (EDC3)	CTGGCTGGCCTTTGATTGCC
qPCA	C Oligo Forward YDL160C (DHH1)	ACAGGCGTATCCTCCACCGC
qPCA	C Oligo Forward YNL147W (LSM7)	TTATAGGTGTCCTAAAAGGC
qPCA	C Oligo Forward YDL160C (DHH1)	ACAGGCGTATCCTCCACCGC

qPCA	C Oligo Forward YGR178C (PBP1)	AGCGAACGGGTCGGCAATGC
qPCA	C Oligo Forward YIR001C (SGN1)	AAAAACACTTCAACAGTGCC
qPCA	C Oligo Forward YNL016W (PUB1)	ACAGCAGCAGCAACAGGGCG
qPCA	C Oligo Forward YNL121C (TOM70)	ATTACTTTTGCTGAAGCCGC
qPCA	C Oligo Forward YML086C (ALO1)	AGGATTTGAAAAAGTTCCGG
qPCA	C Oligo Forward YGL019W (CKB1)	GATGAGGCAGTATCTGGTCC
qPCA	C Oligo Forward YOR061W (CKA2)	ATTAGCTGTTCCTGAAGTGG
qPCA	C Oligo Forward YPR072W (NOT5)	AATCTGAGGAGGAATCATGG
qPCA	C Oligo Forward YER068W (MOT2)	TAAGGTTCCTATTCAGCAGC
qPCA	C Oligo Forward YFR024C-A (LSB3)	ACCATTCAGAAAGGGTGACG
qPCA	C Oligo Forward YOR042W (CUE5)	GAACCCCTGGATACTACACC
qPCA	C Oligo Forward YGL087C (MMS2)	ACTGGAAAAGAGCCTACACC
qPCA	C Oligo Forward YMR140W (SIP5)	CGAACTTGAAGATCAAATGG
qPCA	C Oligo Forward YGL153W (PEX14)	GATAGCAACGCCTCCATTCC
qPCA	C Oligo Forward YNL214W (PEX17)	TTAACAGATAGGTCCCGAGC
qPCA	C Oligo Forward YBL007C (SLA1)	TTACAGAACCAACCTACTGG
qPCA	C Oligo Forward YNL084C (END3)	GTCGATAACTGATGACTTGG
qPCA	C Oligo Forward YLR200W (YKE2)	ATGCGAAAAGAACATAAGGG
qPCA	C Oligo Forward YML094W (GIM5)	TTCCTTGTCCATCGAGGCCC
qPCA	C Oligo Forward YHR012W (VPS29)	TAATTCACCAAGTTTCTGCC
qPCA	C Oligo Forward YJL154C (VPS35)	CACCAACTGAAGTATATCCC
qPCA	Oligo Reverse to test DHFR integration	CCATCTTTTCGTAAATTTCTG
PKA	BCY1-DHFR integration Forward	TGCAGTAGACGTATTAAAGCTCA ATGATCCTACAAGACATGGCGGT GGCGGATCAGGAGGC
PKA	BCY1-DHFR integration Reverse	AGGAAATTCATGTGGATTTAAG ATCGCTTCCCCTTTTTACTTCGA CACTGGATGGCGGCGTTAG
PKA	TPK1-DHFR integration Forward	TCAAGGTGAAGACCCATATGCTG ATCTTTTCCGGGACTTCGGCGGT GGCGGATCAGGAGGC
PKA	TPK1-DHFR integration Reverse	AATATAGATACGAGAGGAAAAT ACAACAAAACATTAGTCATTCGA CACTGGATGGCGGCGTTAG
PKA	TPK2-DHFR integration Forward	TCAAGGCGATGATCCATATGCTG AATACTTTCAAGATTTCGGCGGT GGCGGATCAGGAGGC
PKA	TPK2-DHFR integration Reverse	GTACTTGAAAATTGTTTTTGTGT TTTTTGGTTCATGGAACTTCGAC

		ACTGGATGGCGGCGTTAG
PKA	C Oligo Forward BCY1	GTGATCAAGGGGAGAACTTTTATTT
PKA	C Oligo Forward TPK1	CGACTCTAACACGATGAAAACCTAT
PKA	C Oligo Forward TPK2	GGTATCGGTGACACGTCT
CoIP	BCY1-HA Forward	TACTGGGTCCTGCAGTAGACGTATTAAAGCTCAATGATCCTACAAGACATCGTACGCTGCAGGTCGAC
CoIP	BCY1-HA Reverse	AAGAGAAAGGAAATTCATGTGGATTTAAGATCGCTTCCCCTTTTTACTTAATCGATGAATTCGAGCTCG
CoIP	TPK1-MYC Forward	ACTACGGTGTTCAAGGTGAAGACCCATATGCTGATCTTTTCCGGGACTTCCGTACGCTGCAGGTCGAC
CoIP	TPK1-MYC Reverse	AAAAAAAAATATAGATACGAGAGGAAAATACAACAAAACATTAGTCATTAATCGATGAATTCGAGCTCG
CoIP	TPK2-MYC Forward	ATTATGGTATTCAAGGCGATGATCCATATGCTGAATACTTTCAAGATTTCCGTACGCTGCAGGTCGAC
CoIP	TPK2-MYC Reverse	AGAGAAAGTACTTGAAAATTGTTTTTGTGTTTTTTGGTTCATGGAACTTAATCGATGAATTCGAGCTCG
CoIP	Oligo Reverse to test MYC or HA integration	CGACAGTCACATCATGC
K_d	Oligo used to check Ras and RBD's plasmids constructions	CAACATTTTCGGTTTGTATTAC
K_d	Oligo Forward to amplify DHFR F[1,2] and clone in p413Gal1-Ras contain a restriction site BspEI	ATCGCAGGCTCCGGAGGTGGAGGTTCTGGAGGTATGGTTCGACCATTGAACTGC
K_d	Oligo Reverse to amplify DHFR F[1,2] and clone in p413Gal1-Ras contain a restriction site Xho1	CGATGCCCGCCCCCGCTCGAGCTATGTTCTAGATTAGGTACCCAA
K_d	Oligo Forward to amplify DHFR F[3] and clone in p415Gal1-RBD contain a restriction site BspEI	CGTTGAGGCTCCGGAGGTGGAGGTTCTGGAGGTATGAGTAAAGTAGACATGGTT
K_d	Oligo Reverse to amplify DHFR F[3] and clone in p415Gal1-RBD contain a restriction site Xho1	AGATCGCCGCCCCCGCTCGAGCTAAGTTCTAGATTAGTCTTTCTT

C Oligos were used to confirm the integration at the proper locus.

Supplementary Figures



Electronic Supplementary Material (ESI) for Molecular BioSystems

Fig. S1. Dynamics of the interactions between the PKA regulatory and catalytic subunits in response to different perturbations. (A) Comparison of the DHFR-qPCA signal for the Bcy1-Tpk1 interaction in glucose and galactose (B) DHFR-qPCA signal for the interaction Bcy1-Tpk1 in cells grown in media supplemented with caffeine at different concentrations. (C) DHFR-qPCA signal for the Bcy1-Tpk1 interaction in cells grown in media supplemented with methyl methanesulfonate. (D) DHFR-qPCA signal for the Bcy1-Tpk1 interaction in cells grown in media supplemented with galactose and methyl methanesulfonate at different concentrations. (E) DHFR-qPCA signal for the interaction Bcy1-Tpk1 in strains carrying an additional copy (on a low copy number plasmid) or a deletion of one copy (heterozygous strain) of the genes coding for the PDE enzymes (left and right panel, respectively). In all cases, n represents the number of independent replicates.

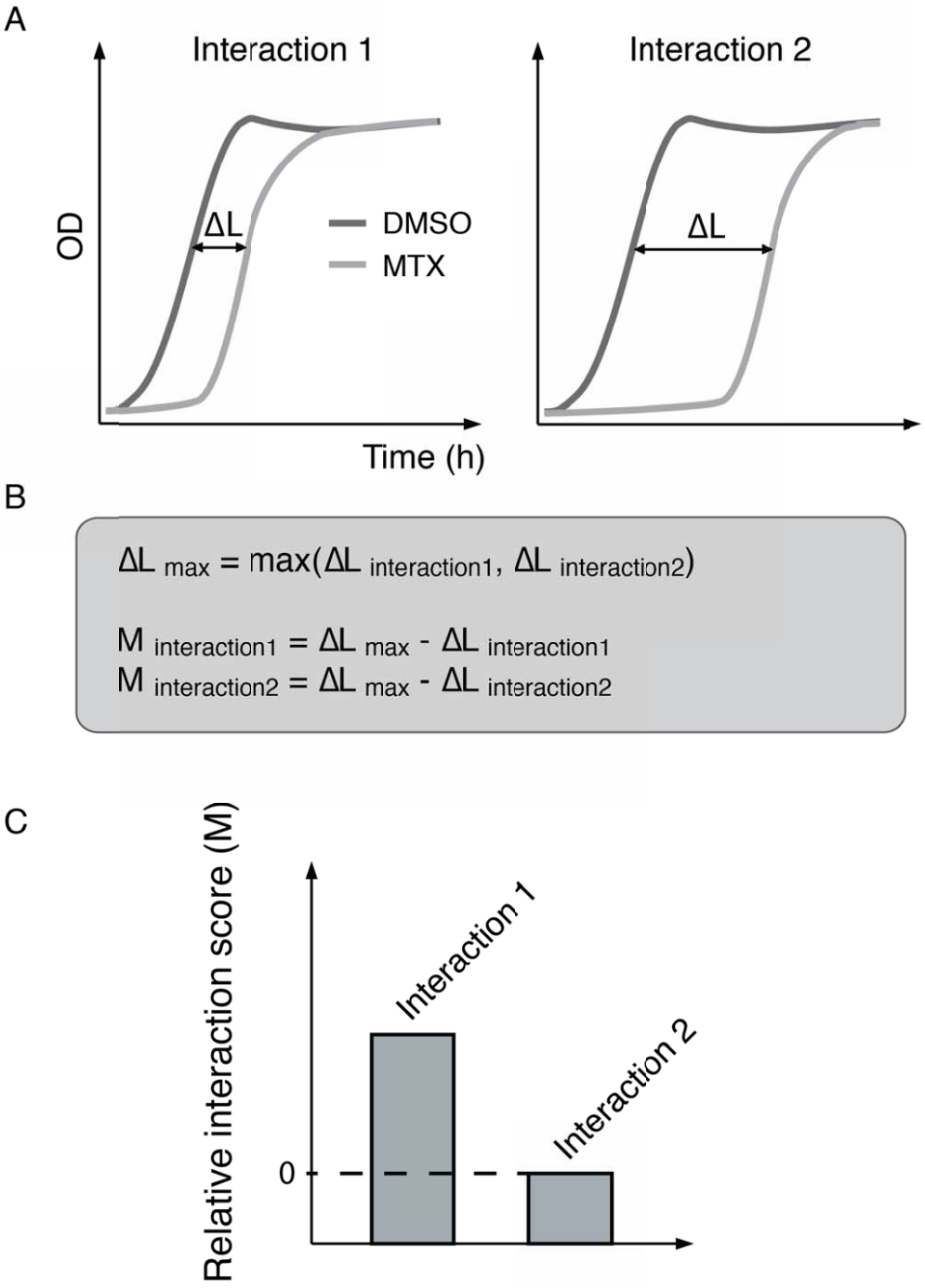


Fig. S2. Comparing PPIs using the M relative interaction score. (A) The difference between the lag times in DMSO and MTX (ΔL) is calculated for all interactions.
(B) M scores are calculated for each interaction by subtracting to the maximum ΔL of all interaction the ΔL of a specific interaction. (C) Bar graphs are generated to compare the relative interaction scores of all interactions tested.