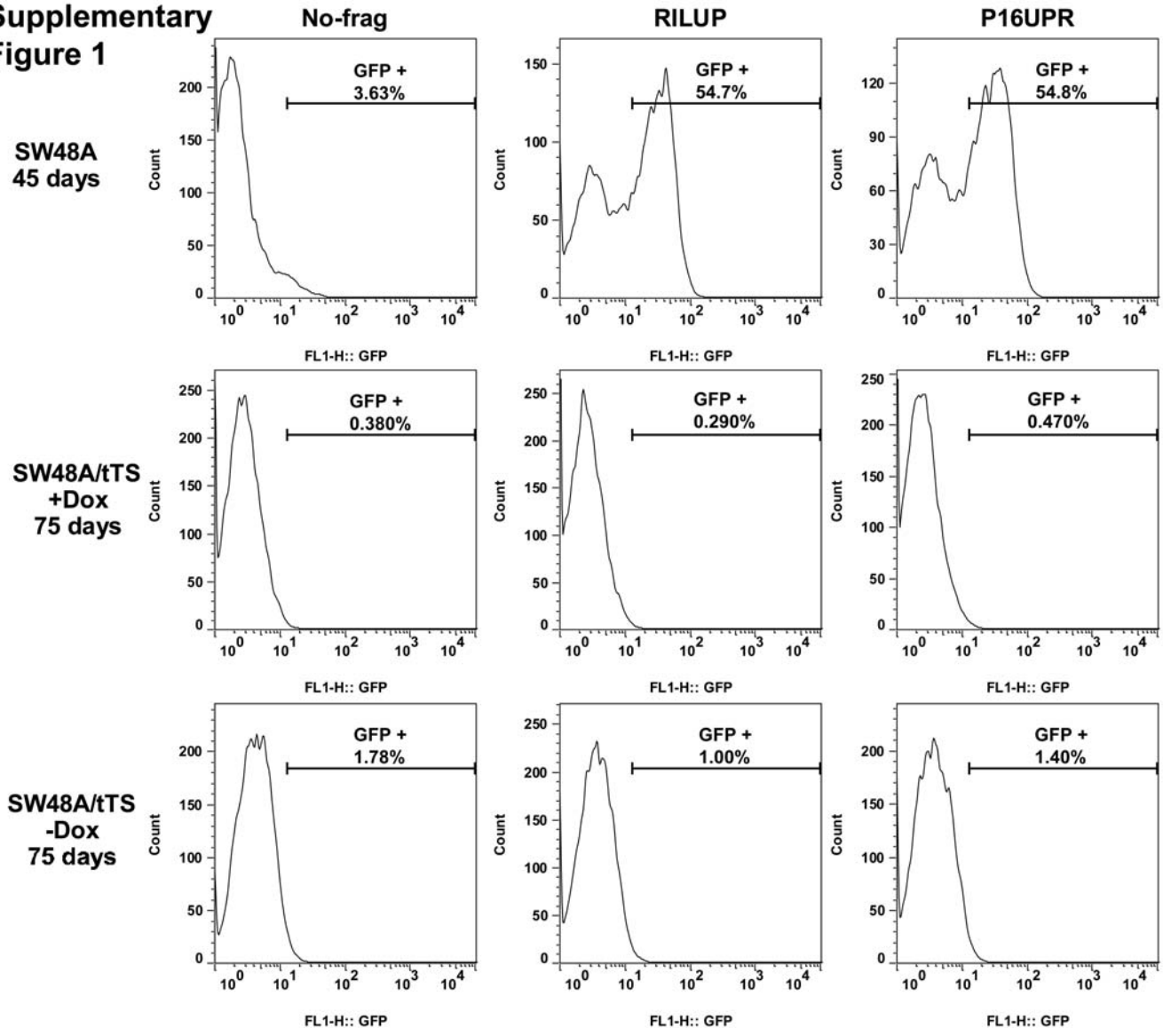
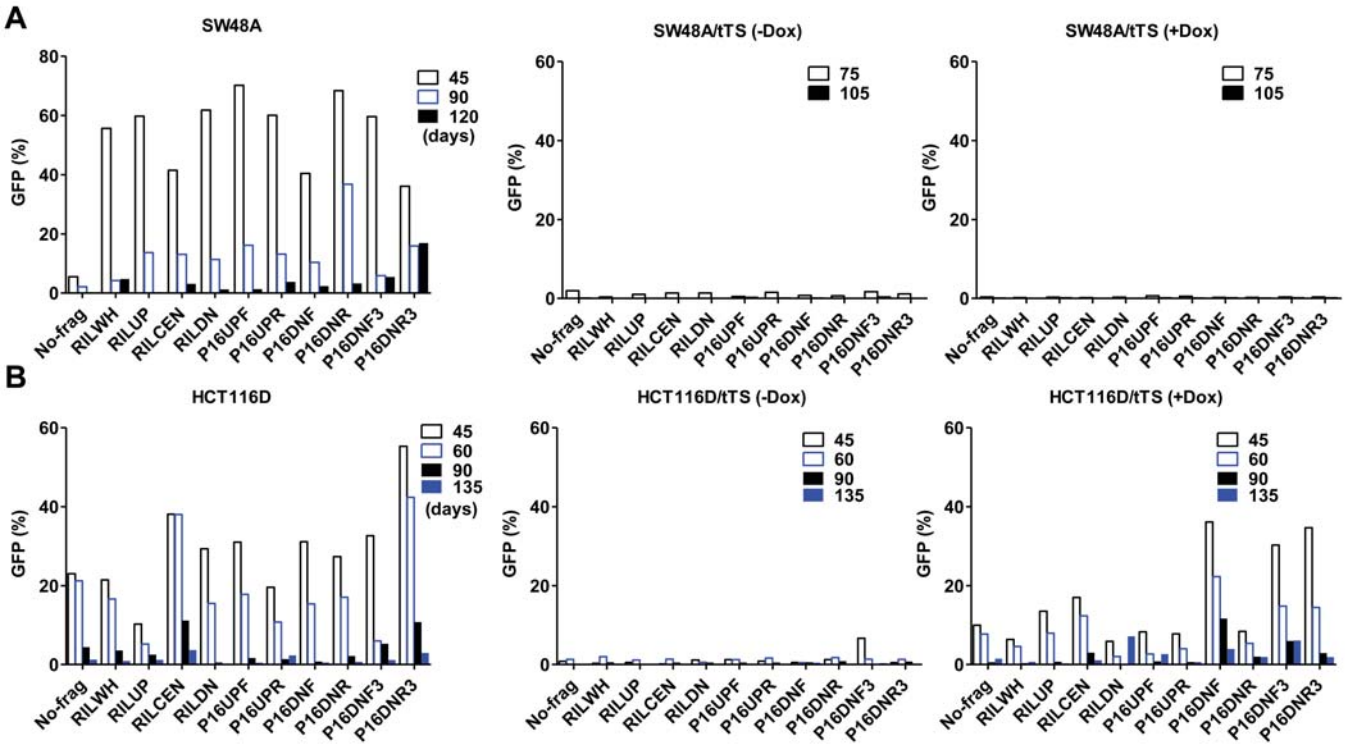
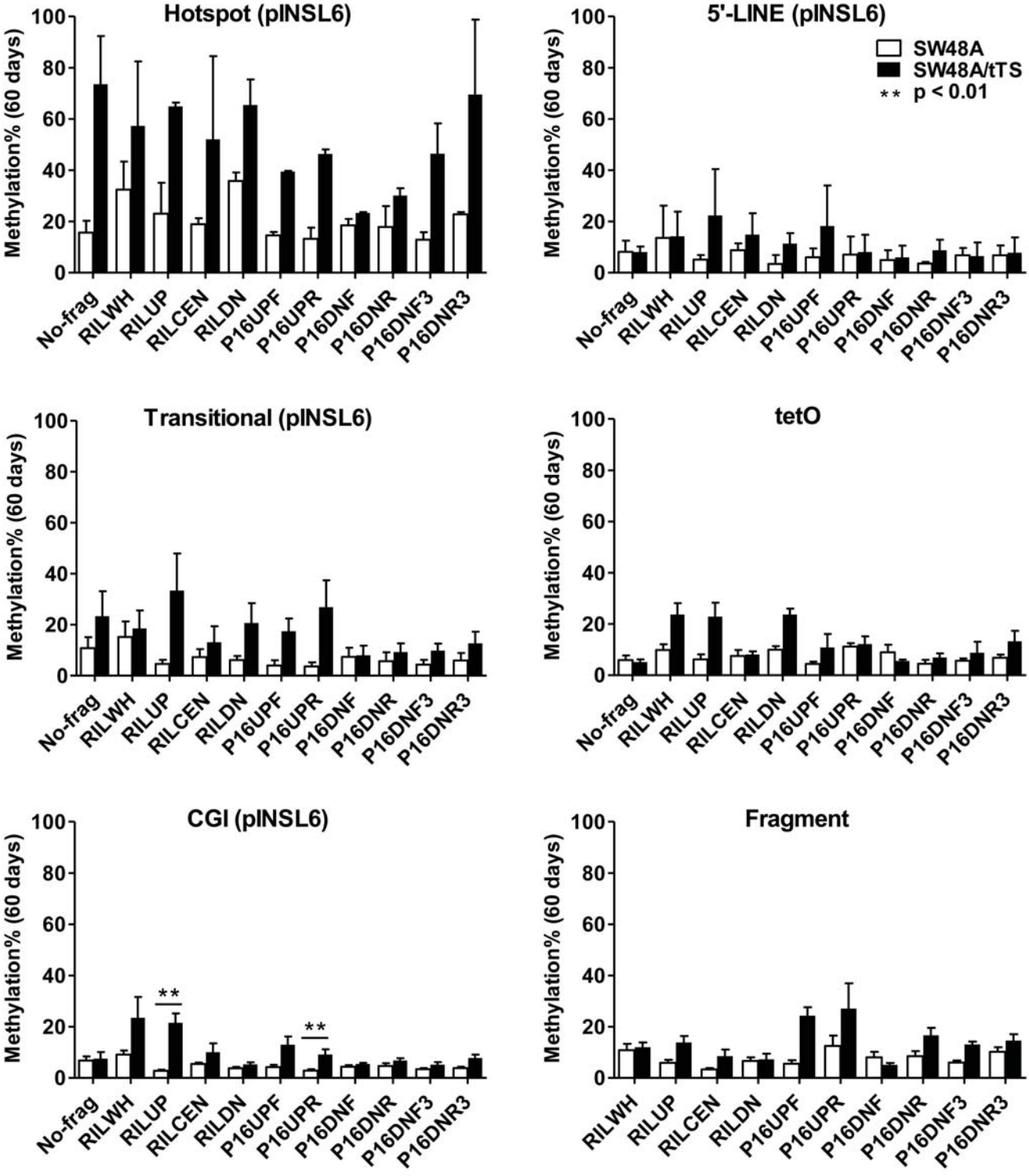
Supplementary Figure 1



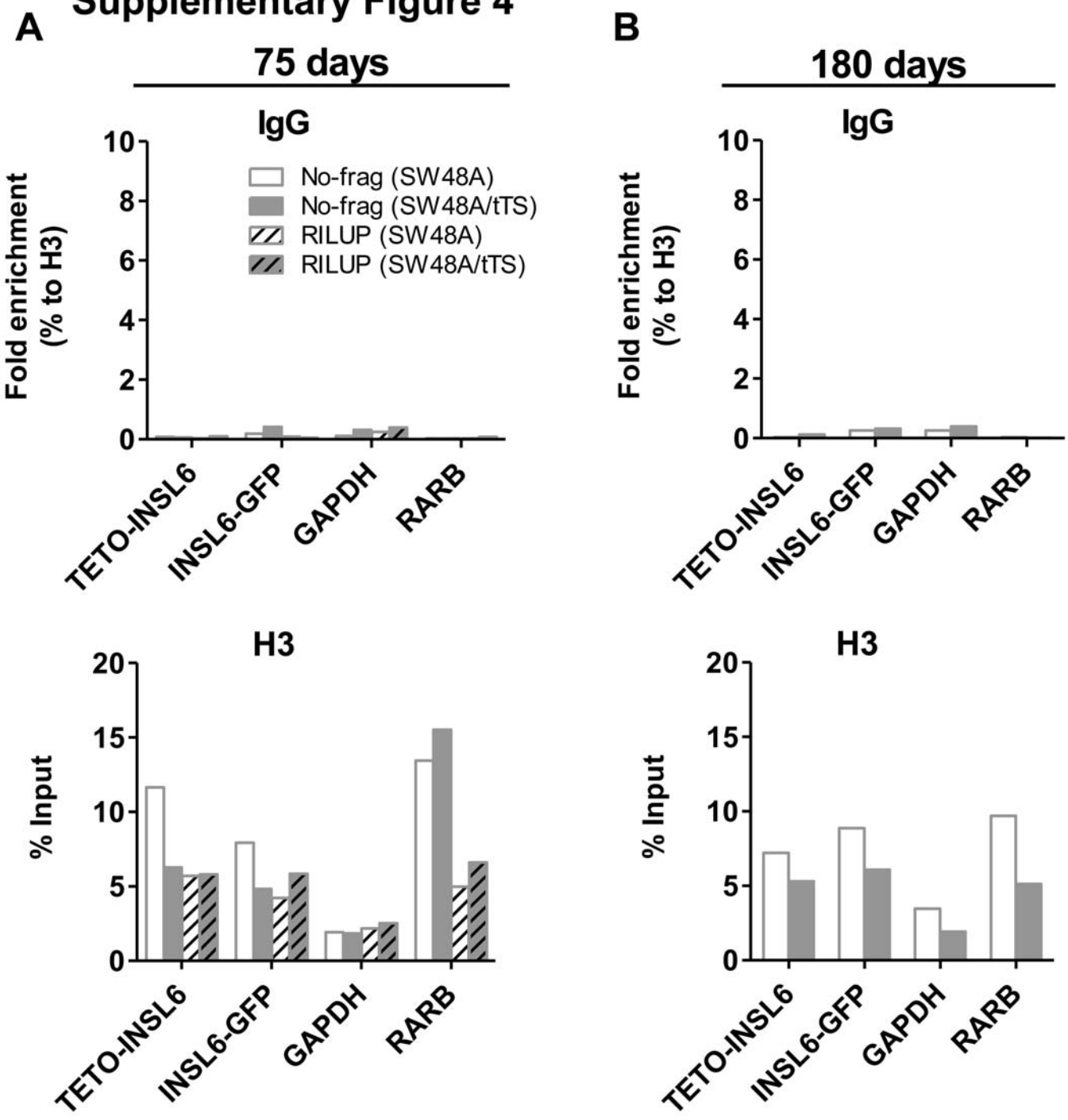
Supplementary Figure 2



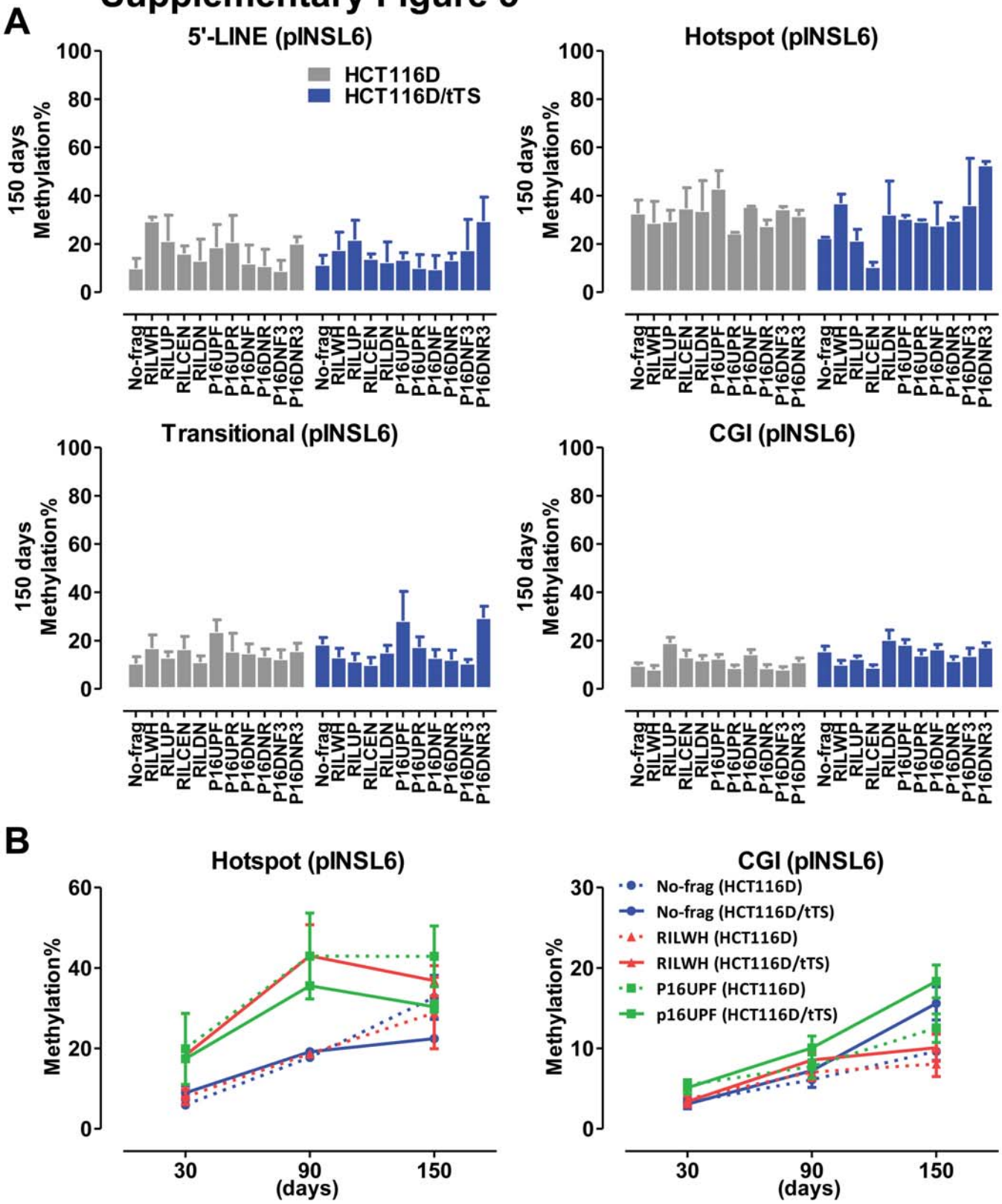
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary figure 1. Flow cytometry graphs to exemplify transgene expression in Flp-in or Flp-in/tTS cells at the earliest available time points (45 days for SW48A and 75 days for SW48A/tTS). Three transgenes (No-frag, RILUP and P16UPR) are shown here, and please refer to Supplementary Fig. 2 for more transgenes and other time points.

Supplementary figure 2. The function of tTS in accelerating GFP silencing. GFP expression was measured by flow cytometry. Stable single clones were sampled at several time points from 45 days to 135 days after transfection. For Flp-in/tTS host cells, clones were cultured in media with and without doxycycline (Dox) respectively. (A) SW48A and SW48A/tTS as host cells; (B) HCT116D and HCT116D/tTS as host cells. GFP expression in long-term cultured tTS-containing clones presented partial inducibility in response to doxycycline, and maintaining clones under doxycycline did not prevent GFP from silencing in the long run.

Supplementary figure 3. Comparing the methylation profiles of pINSL6 in SW48A/tTS and SW48A cells (60 days). **, significantly different methylation of each transgene between host cells ($p < 0.01$).

Supplementary figure 4. ChIP-qPCR to analyze the H3 occupancy and IgG affinity in pINSL6. All the values of fold enrichment were normalized to H3 and H3 occupancy was calculated as the percentage of input. GAPDH and RARB as the control regions. TETO-INSL6 and INSL6-GFP are targets designed at the 5'-end and 3'-end of pINSL6 in order

to distinguish it from the endogenous one. (A) Two transgenes (No-frag and RILUP) examined in SW48A and SW48A/tTS at 75 days. (B) The transgene (No-frag) examined in SW48A and SW48A/tTS at 180 days.

Supplementary figure 5. Methylation profiles of pINSL6 of transgenes in HCT116D and HCT116D/tTS. Pyrosequencing was used to detect regional methylation patterns as described before. (A) Methylation in HCT116D/tTS and HCT116D, sampled at 150 days. Error bar, SEM. (B) Gradual accumulation of transgene methylation. Three time points (30, 90 and 150 days) are shown for three transgenes (No-frag, RILWH and P16UPF).

Supplementary table 1. PCR primers and sequencing primers used for bisulfite-sequencing and pyrosequencing.

Name	Step	Forward	Reverse	Sequencing	Target Sequence	Ta (°C)	Size (bp)
BSP	1°	GGTAYGATGGAAAGTAAGAATAG	TCCAACTCRACCAAAATAAACAC	NA	NA	50-58(53)	642
INSGFP-P1	1°	GGTAYGATGGAAAGTAAGAATAG	TCCAACTCRACCAAAATAAACAC			53	642
	2°	GTAAGATGGAAAGTAAGAATAGA	U-AAAAACCCCCTATCATCTCT	TAAGTTTATAAATGGAAATA	GGTYGATTAATATYGTGAAA	50-61(58)	128
				AGGTTTTTGTTTTTATGAAG	YGGGGGTTTTAGGAAAGAGGTTATAYGTTTAT		
INSGFP-P2	1°	GGTAYGATGGAAAGTAAGAATAG	TCCAACTCRACCAAAATAAACAC			53	642
	2°	*U-TAGGAGTAGATAGGGAGTAGG	CTCCACCAAAATAAACAC	AACCCCAAACTAATCCT	CCRCRTTATACAATAAC	50-61(58)	200
INSGFP-P3	1°	GGTAYGATGGAAAGTAAGAATAG	TCCAACTCRACCAAAATAAACAC			53	642
	2°	U-TGAGGGAGAAGAAGTTTTATTAA	CCCCCTAATTTAATTCATTT	TCCCTCCACCTAAAATAC	RTTTCTCRTTCTTATTTA	54	174
				AATAAAAAATTCCTCACCA	AACRTAAAAACA		
INSGFP-P4	1°	GGTAYGATGGAAAGTAAGAATAG	TCCAACTCRACCAAAATAAACAC			53	642
	2°	U-TTTTGGTTTTTATTTTAGGAGTTAG	CCCCTTACTCACCATAATAAC	CCAACCCTACTCCCTATC	TACRCCTACRCCAAACCRAACRACRCRCAC	50-62(58)	329
tetOINS-P1	1°	TTYGGGATTTGGTATTTAGTTG	CCGGAAACTCATACCCTAAACTC			50-58(55)	707
	2°	AGGGATTTGGTATTTAGTTGGTAATT	U-ACCCATACCTCCCTATAAACCAATAA	TGGTAATTAAATATGTGGTG	AAAYGTTTAAGTGTTGTATATGGTTATGTGYGTTTAGTTA	50-62(58)	129
tetOINS-P2	1°	TTYGGGATTTGGTATTTAGTTG	CCGGAAACTCATACCCTAAACTC			50-58(55)	707
	2°	TTGAGGTATTTGGGATGGAAATT	U-CAAATCCTCTAAAAATCCCTATCACA	GGATGGAAATTTGGTTTT	TTYGGTTAGATAA	50-62(58)	99
tetOINS-P3	1°	TTYGGGATTTGGTATTTAGTTG	CCGGAAACTCATACCCTAAACTC			50-58(55)	707
	2°	GATAATTTTGTTTAGTTAGGA	U-AAACTCATACCCTAAACTC	TTTATTATTGATAGGGAGTAAAT	TYGATATAYGTTTTTTATTA	50-58(58)	390
RILWH-P1	1°	ATGAAGAATTTGTTTAGGGTTAGG	U-AAACATTCAAACCTTCTCTTACTACTTA	AGATGTAAGGGTTAGATATA	YGYGTTGATATTGATTATTG	50-58.9	198
RILWH-P2	1°	GTTTAGTGTGGTGGAATTTTGTAG	AACCRCRAACCCAAACCCAAC			58-62(58)	487
	2°	GGTGGTTTGAGAGGAGTTTGTAA	U-TATCCACACCCACCCTTTA	TTTTTTATAAGGTAGGATGG	GTYGTAGGTGTGTTAGTYGGGTTTAT	50-62(60)	401

			AAATC				
RILUP-P2	1°	TAGTGTGGTGGAATTTTGTAGA	CACTATACTAAAAACTACCCCAA			52-62(55)	394
	2°	AGAAGGTTTGAATGTTTTGGGGAAGTA	U-TACCCCAACTCAAATACCTCCTCATAA	TTTTTTATAAGGTAGGATGG	YGYGTTGATATTGATTATTG	50-62(58)	286
RILCEN-P	1°	GTTTAGTGTGGTGGAATTTTGTAG	ACCCTCCCACTTCRAAAAACTCT			50-62(55)	319
	2°	AGGTGTAGATAGTTGGGTTTGGG	U-CACACCCCCACTCAACTCTC	AAGGTTAGAGTAGGATTTAG	GTYGTYGGGGTYGTTYGAAYGYGGGGATTT	50-62(55)	113
RILDN-P1	1°	ATGAAGAATTTGTTTAGGGTTAGG	U-ATCCACAATAACTCCCCCAAATA	AGATGTAAGGGTTAGATATA	YGYGTTGATATTGATTATTG	58	333
RILDN-P2	1°	TAGTGTGGTGGAATTTTGTAGA	CRAAAACCCCAAACCTCCCTAAA			58	421
	2°	U-TTTGGGGGAGTTATTGTGGAT	CAAAACCCCAAACCTCCCTAAAT	CCCCTAACCCATCTC	CRCRAATCCTTCCCRAATCCAC	60	196
P16UPF-P1	1°	ATGAAGAATTTGTTTAGGGTTAGG	U-TAAACACCCTCCACTAATCA	AGATGTAAGGGTTAGATATA	YGYGTTGATATTGATTATTG	58	96
P16UPF-P2	1°	TGAAGAATTTGTTTAGGGTTAGG	ATCTCTCCACCACCCTCC			50	548
	2°	TTTTAGGTTGGAGTGTAATG	U-CTCTATAATCCCAACATTCT	GTAGTTGGGATTATAGGTAT	GYGTTATTAAGTTTYGTTAATTTTG	50-55	243
P16UPF-P3	1°	TGAAGAATTTGTTTAGGGTTAGG	ATCTCTCCACCACCCTCC			50	548
	2°	YGTTATGTTGGTTAGGTTT	U-ATCTCTCCACCACCCTCCA	TTAGAATGTTGGGATTATAG	AYGTGAGTTATYGTATTYGGATTTT	52-58	207
P16UPR-P1	1°	ATGAAGAATTTGTTTAGGGTTAGG	U-ATTCCCTTCCCCCTTTATAATTAC	AGATGTAAGGGTTAGATATA	YGYGTTGATATTGATTATTG	58	166
P16UPR-P2	1°	TGAAGAATTTGTTTAGGGTTAGG	CACACRTACRCCACCATAACCAA			50	501
	2°	GGGGTTGTTGTGAGTTTAAATGAT	U-TACCCCACCATAACCAACTAATT	AATTTTAGTATTTTGGGAAG	TYGAGGYGGGTAGATTATTTGAGG	50-55	306
P16DNF-P1	1°	ATGAAGAATTTGTTTAGGGTTAGG	U-ACCACAAATTTCCACTAATAATCC	AGATGTAAGGGTTAGATATA	YGYGTTGATATTGATTATTG	58	114
P16DNF-P2	1°	TGAAGAATTTGTTTAGGGTTAGG	CTAAAACATAAACTCCAACCAC			58	601
	2°	GGAGGGATTATTAGTGGAAATTTG	U-TTCTTTCCACAAAATCTCACTCTA	AGGTTGAGGTAGGAGAAT	YGTTTGAATTYGGGAGGTTG	60	370
P16DNR-	1°	ATGAAGAATTTGTTTAGGG	U-	AGATGTAAGGGTTAGAT	YGYGTTGATATTGATTAT	58	466

P1		TTAGG	CCCTTATAATCCCTTCACTTTAAA	ATA	TG		
P16DNR-P2	1°	AATTTYGGTTYGAGTTTAGGGTAT	TCAAACRATTCTCCTACCTCAACC			58	335
	2°	GTGTTGGATATTAGGAGGGATTAT	U-CCACCCCCCACTACTTTTTATAT	TGATAGAAATTATTTAGAAG	YGGTYGGGYGYGGTGTTTTAYGTTTTGTAA	58-62	241
EGFP-P	1°	AAGGAAGAAGGTAATTATAAGA	U-CCAACTTATACCCCAAAAT	GGAAGAAGGTAATTATAAGA	TTYGYGTYGAGGTGAAGTTYGAGGGYGATAT	50-52	124
HYG-P	1°	AGGGTGTTAAGTTGTAAGATT	U-TCCCCCATATAAAATCAC	AATTGTTAGTTGTTTTGTAG	TYGGTYGYGGAGGTTATGGATGYGATYGTTGYGGTYGATTTT	50-52	174
siteD-P1	1°	GTAAGAGTAGAAATAGGGGAAGA	U-CATCTCCCTTAACTTCACAA	AATTAGTTTTTTTTTTATGA	YGGAGTAAGGATGGAGAATAAGGATATYGGTTTA	50-58	277
siteD-P2	1°	AGAGATGTAGTAGGTAGTTGGATATA	U-AATTAAAACCAATACTTACATATTC	TTTTATAGTATAAGTTTATAGGG	TAYGAAGAATGAATTTTGAGAATGTTTGG	50-58	75
siteA-P1	1°	AGTGTATTTGGGGATAAGA	U-TCTTTCACCACTTAATTAATC	TTATGATAGATATTTGGTAA	AYGATGGAAATGT	50-52	78
siteA-P2	1°	AGTTTAGGTTTTTGGAATGTAG	U-TTATTTACTCACCTTCTTTACAT	TGTATTTAGGTTAATTTATG	TTGTATTTGAATTYGAYGTTAA	50-52	243
Zeo-P1	1°	AAGTAGTGATTGGGAAAAT	U-CATTCCCCATTCAAACTA	ATTGGGAAAATTTTGG	YGTTATTTAATTTAATYGTT	50	138
Zeo-P2	1°	GGTTGATTAATTTTTTTTATTTATGT	U-AAACTTCCAAATAATAACTTCTC	TTTTTTTTATTTATGTAGAG	GTYGAGGTYGTTTYGGTTT	50-52	120

*U-: Biotin-labeled universal primer (GGGACACCGCTGATCGTTTA).

Supplementary table 2. Realtime-PCR primers and probes used for ChIP analysis.

Name	Forward	Reverse	Probe	Amplicon(bp)
SiteA	TGCTGTATTTGAATCCGACGTT	GCCTTGGATGGAAGAACAAATC	ATCTGAAAGGAATCCCT	69
SiteD	AACTTCACAAACATATACACTCTGCTGAT	GACGGAGCAAGGATGGAGAA	CTAGACCGGTGTCCTT	67
TETO-INSL	GGCGTGTACGGTCATACTTAAGG	GGTGAGATAATTTTGCTCAGTTAGGA	TCTCTCGAGCCCCTTC	85
INSL-GFP	GCCGACCGCCATTGC	ACTGCAGAATTCGAAGCTTACTTAGA	TCACAGGAGATCTGC	79
ACTB	TCCCCTCCTTTTGCGAAAA	CGGCCAACGCCAAAACT	AGCGAGATTGAGGAAGA	78
GAPDH	CTGCTCCTCCTGTTCGACAGT	TCACCTGGCGACGCAAA	AGCCGCATCTTC	52
RARB	TTGGAAGGAGAACTTGGGATCTT	AGGCTTGCTCGGCCAATC	CTGGGAACCCCCC	85

Supplementary table 3. Fragments subcloned into transgenes.

Fragments	Distance to TSS (Gene)	Subcloned length (bp)	CG sites	Orientation
RILWH	-713 to +664 (RIL)	1421	96	Forward
RILUP	-713 to -378 (RIL)	336	11	Forward
RILCEN	-406 to +237 (RIL)	643	66	Forward
RILDN	+214 to +664 (RIL)	451	20	Forward
P16UPF	-1752 to -804 (P16)	980	33	Forward
P16UPR	-1752 to -804 (P16)	980	33	Reverse
P16DNF	+768 to +1248 (P16)	511	18	Forward
P16DNR	+768 to +1248 (P16)	511	18	Reverse
P16DNF3	(+768 to +1248)x3 copies (P16)	1485	18x3	Forward
P16DNR3	(+768 to +1248)x3 copies (P16)	1485	18x3	Reverse