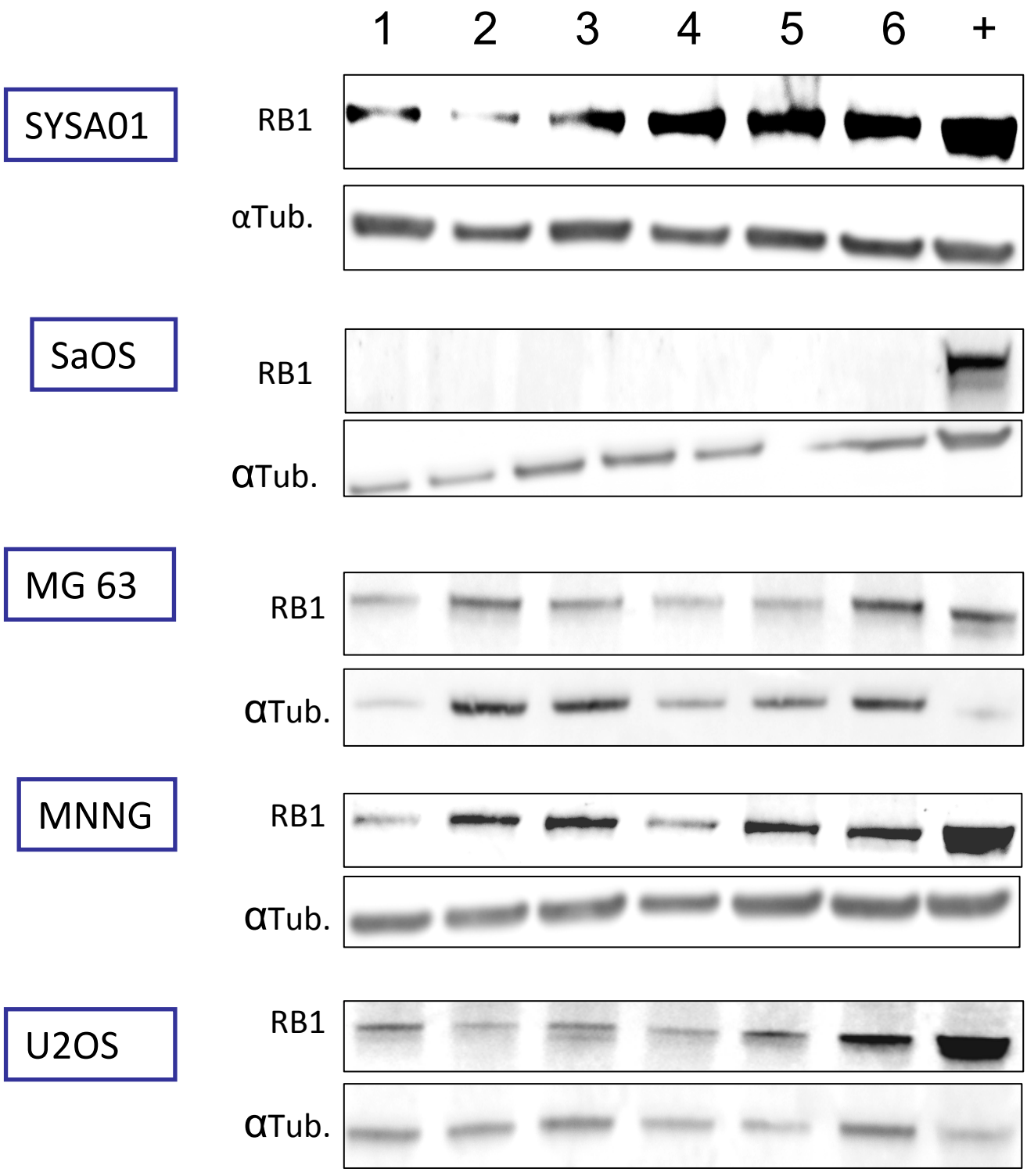
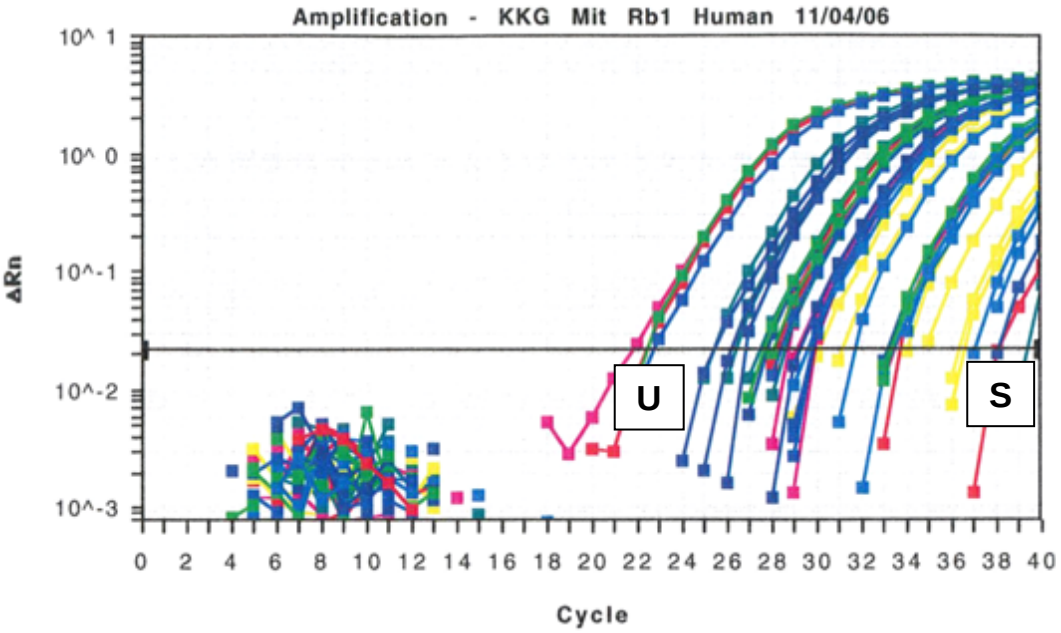


S8a: RB1 protein expression in human osteosarcoma cell lines

20µg protein extracts from 5 unirradiated (1-3) or 4Gy gamma-irradiated (4-6) human OS cell lines were probed on western-blot for RB1 (mAB G3-245, BD Pharmingen) and α-Tubulin as loading control. Protein extracts were harvested 0h (1,4), 2h (2,5) or 4h (3,6) after treatment. + Positive control: protein extracts from HEK293 cells.

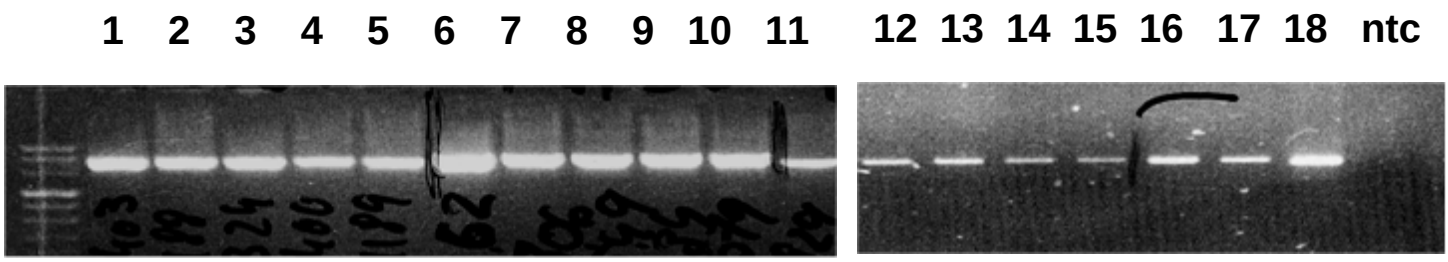


Case	Rel. mRNA
OS-1	2,17
OS-2	0,25
OS-3	3,66
OS-4	1,58
OS-5	0,64
OS-6	0,17
OS-7	1,02
OS-8	1,04
OS-9	0,34
OS-10	0,09
OS-11	0,69
OS-12	4,36
OS-13	0,21
OS-14	0,27
OS-15	0,20
OS-16	1,44
OS-17	0,32
OS-18	0,52
OS-19	0,68
OS-20	0,17
OS-21	0,19
SaOS	0,00
U2OS	1,11
Cal	1,00

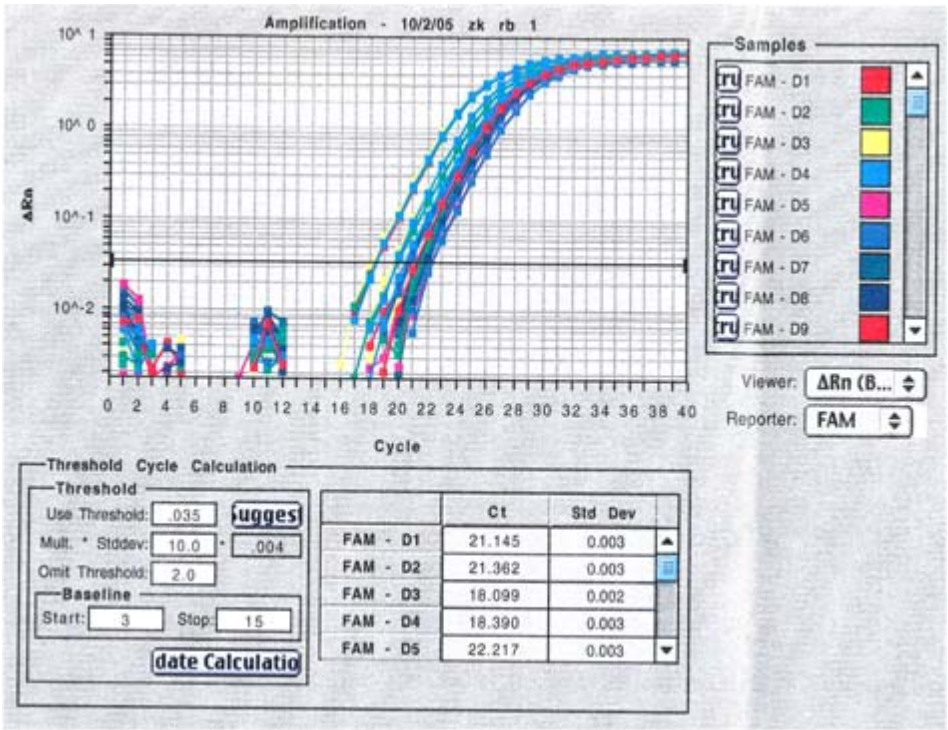
S8b: RB1 mRNA Expression in human osteosarcoma

RB1 cDNA amplification plot and rel. mRNA expression of 21 primary human Osteosarcoma cases in comparison with cell line U2OS (**U**, expressing Rb1) and SaOS (**S**, Rb1 deficient).

After reverse transcription using SuperSript III (Invitrogen) cDNA from tumors and cell-lines were amplified using the real-time quantitative system (Taq.Man) ABI PRISM 7700 SDS (Applied Biosystems) for RB1 (probe Hs00153108_m1 and TBP (probe Hs00427620_m1).
Expression values are relative to TBP and normalized to a Calibrator (pooled human cell lines)



mRb1 Rt-PCR using primers Ex2f-363 and Ex11r-1231 to amplify a 887 bp long fragment from cDNA derived from murine osteosarcoma cell lines 1403, 1188, 1324, 1400, 1189, 62, 306, 549, 734, 679, 1929, 182, 733, 734 (1-11,16-18) and total embryo mRNA from CBA/CA (12-15)
+ no template control.



Cell Line	Rb1 rel to TBP to embryo
MOS62	0,32
MOS162	0,68
MOS184#15	0,46
MOS184#20	0,39
MOS306	0,27
MOS549	0,22
MOS679	0,11
MOS733	0,14
MOS734	0,06
MOS1189	0,98
MOS1324	0,18
MOS1400	0,15
MOS1929	0,23
MC3T3	0,09
Whole Embryo	1,00
ntc	<< 0.0001

S8c: Rb1 mRNA expression in murine osteosarcoma cell lines

Rb1 cDNA amplification plot and rel. mRNA expression of 13 murine Osteosarcoma cell lines, mesenchymal cell line MC3T3 in comparison with whole-embryo mRNA.

After reverse transcription using SuperSript III (Invitrogen) cDNA was amplified using the real-time quantitative system (Taq.Man) ABI PRISM 7700 SDS (Applied Biosystems) for mouse *Rb1 (*probe Mm01310561_m1) and *Tbp* (probe Mm00446973_m1). *Rb1* Expression values are relative to TBP and normalized to a Calibrator (whole embryo).