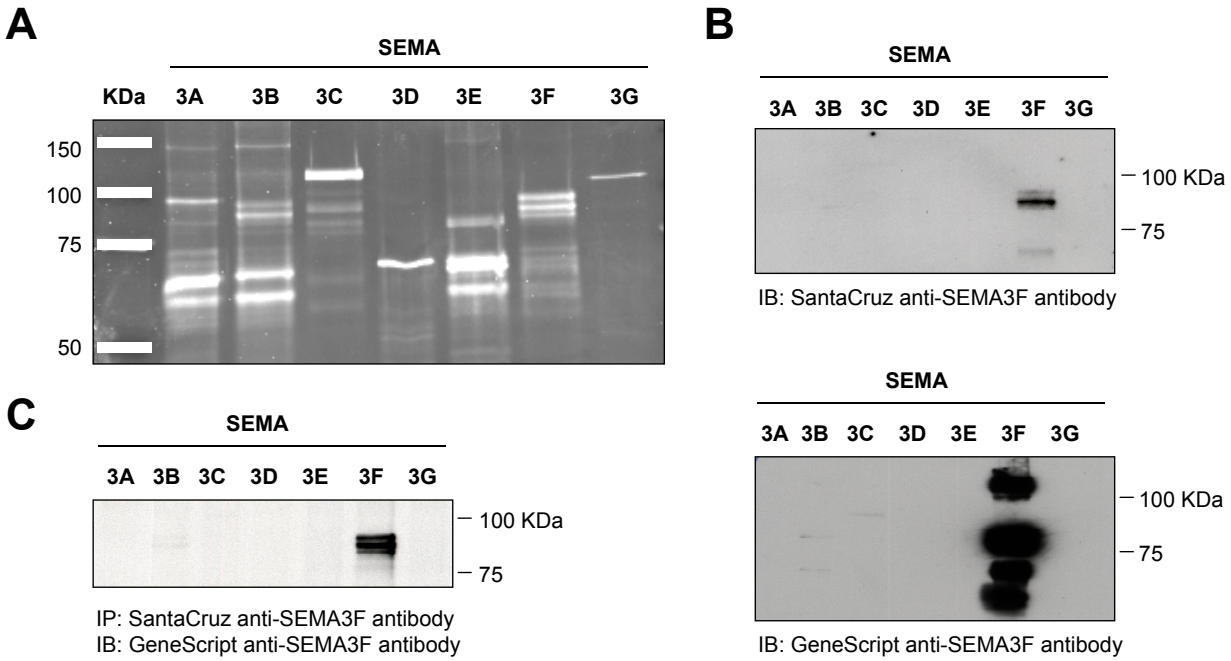
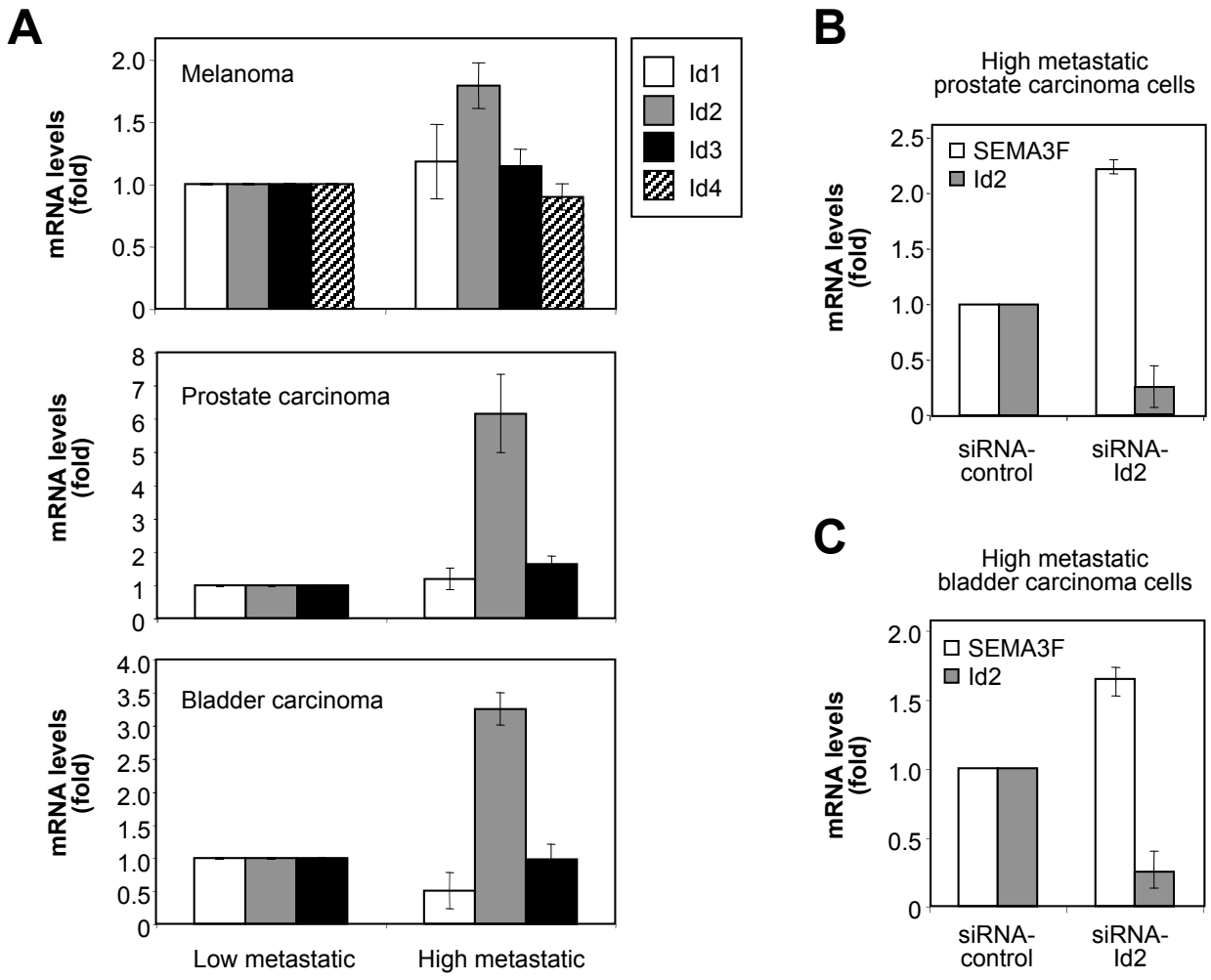
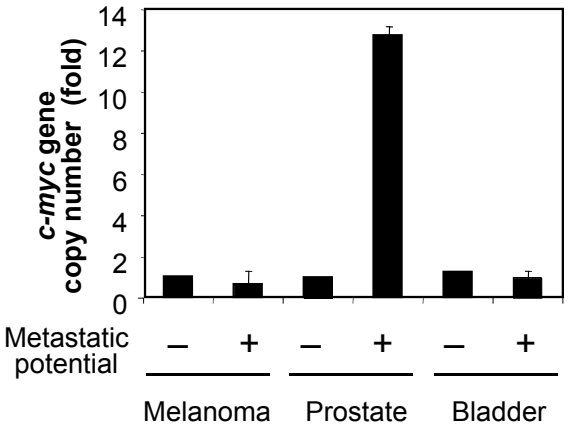
Supplementary Figure S1. Specificity of anti-SEMA3F antibodies. **(A)** One μg of purified SEMA3A-G proteins were analyzed by SDS/PAGE and SUPRO Ruby protein gel stain (Bio-Rad). SEMA3A, 3B, 3E and 3F were purified as described before (30). SEMA3C was purchased from R&D Systems. SEMA3D and SEMA3G were purchased from Novus Biologicals. **(B)** Immunoblot analysis of 1 μg of purified SEMA3A-G proteins using the Santa Cruz anti-SEMA3F antibody (top panel) or the GeneScript anti-SEMA3F antibody (bottom panel). **(C)** Full media (1.2 ml) containing 200 ng of each of the purified SEMA3A-G proteins were immunoprecipitated overnight at 4°C with the Santa Cruz anti-SEMA3F antibody. Protein G slurry was added and incubated for 1 h at 4°C. Immunoprecipitated SEMA3F was detected by immunoblot with the GeneScript anti-SEMA3F antibody.



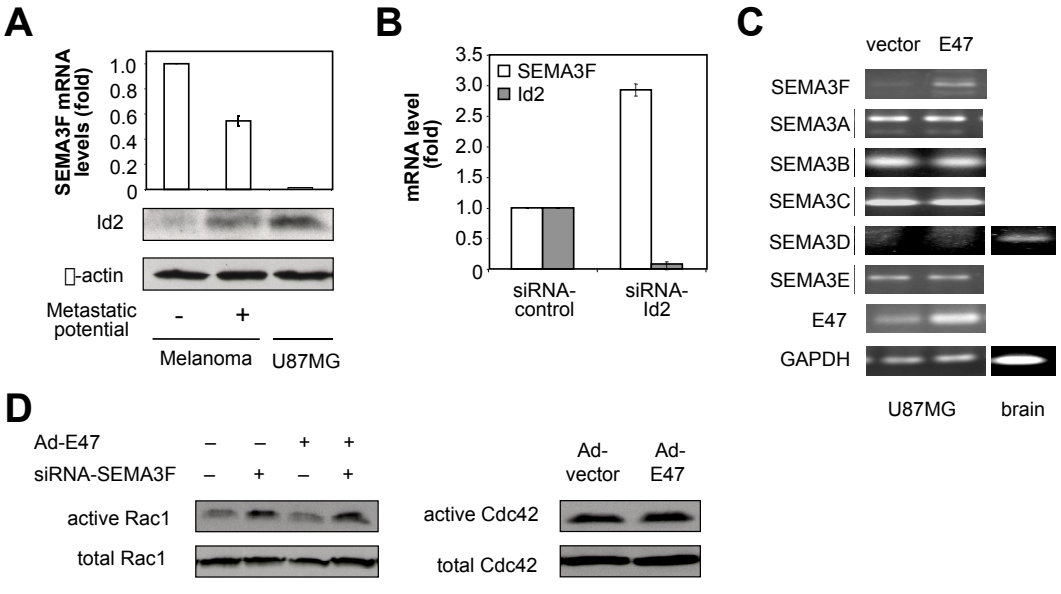
Supplementary Figure S2. **(A)** High metastatic tumor cells upregulate Id2 expression. Id1-4 mRNA levels in paired human cancer cell lines with low (-) versus high (+) metastatic potential. No transcripts for Id4 were detected in prostate and bladder carcinoma cells. **(B, C)** Silencing of Id2 in high metastatic tumor cells induces SEMA3F expression. SEMA3F and Id2 mRNA levels in high metastatic prostate **(B)** and bladder **(C)** carcinoma cells transfected either with siRNA-control or siRNA-Id2.



Supplementary Figure S3. High metastatic prostate carcinoma cells carry an elevated copy number of the *c-myc* gene but high metastatic melanoma and bladder carcinoma cells do not. C-myc copy number of paired human cancer cell lines with low (-) versus high (+) metastatic potential. Genomic DNA was isolated from the cells using the DNeasy tissue kit (Qiagen). *C-myc* gene was amplified by real-time PCR. TOP2B was amplified as a control.



Supplementary Figure S4. **(A)** U87MG glioma cells express high Id2 and low SEMA3F levels. SEMA3F mRNA and Id2 and □-actin protein levels in low and high metastatic melanoma cells and in U87MG cells. **(B)** Silencing of Id2 in U87MG cells induces SEMA3F expression. SEMA3F and Id2 mRNA levels in U87MG cells transfected either with siRNA-control or siRNA-Id2. **(C)** Overexpression of E47 in U87MG cells increases SEMA3F mRNA levels but not expression of other members of the SEMA3 family (SEMA3A-E). SEMA3A-F and E47 mRNA levels in U87MG cells transfected with control or pcDNA3-E47 vectors. **(D)** E47 induces inactivation of Rac1; Cdc42 is not inactivated by E47. U87MG cells were transfected either with siRNA-control (-) or siRNA-SEMA3F (+) and infected either with Ad-vector (-) or Ad-E47 (+). Rac1 (left panel) and Cdc42 (right panel) activities were measured using a Rac1 activation kit (Cytoskeleton, Denver, CO) followed by SDS-PAGE and immunoblot with an anti-Rac1 antibody (Cytoskeleton) or an anti-Cdc42 antibody (Cell Signaling Tech), respectively. Expression levels of total RhoA and total Cdc42 in lysates are shown in the panels below.



Supplementary Table S1. The sequences for primers for real-time RT-PCR.

	Forward	**Reverse**
Human SEMA3F	5'-AGCAGACCCAGGACGTGAG-3'	5'-AAGACCATGCGAATATCAGCC-3'
Human Id2	5'-ATGAAAGCCTTCAGTCCCGT-3'	5'-TTCCATCTTGCTCACCTTCTT-3'
Human c-myc	5'-TCCTCAAGAGGTGCCACG-3'	5'-TCGGTTGTTGCTGATCTGTC-3'
Human □2 microglobulin (B2M)	5'-GAATGGAGAGAGAATTGAAAAAGTGGAGCA-3'	5'-CAATCCAAATGCGGCATCTTCAAAC-3'

Supplementary Table S2. The sequences for primers for semi-quantitative RT-PCR.

	Forward	**Reverse**
Human SEMA3A	5'-AGGACCTTCCTGATGATGT-3'	5'-CAGTGAGTCAGTGGGTCTC-3'
Human SEMA3B	5'-GCAACTGGGCAGGGAAGGACATTG-3'	5'-CCAGTGCGTTGCGCTGCGCGG-3'
Human SEMA3C	5'-AACGCTGCTGATGGGAGATACCA-3'	5'-AACGCTGCTGATGGGAGATACCA-3'
Human SEMA3D	5'-CACTGATTAAGTCCACCCGAGA-3'	5'-TCGCATCTGTGCAAGGAGAGCTG-3'
Human SEMA3E	5'-ACTGCTGCCTGGCTCGAGACCC-3'	5'-CTACTGTCTGGCAAAAATAGGTCC-3'
Human SEMA3F	5'-GGAGCGCAATGATGATAAGC-3'	5'-AATAGTGGTAAGGCGGTAGGG-3'
Human E47	5'-AGTACGGACGAGGTGCTGTC-3'	5'-GCTTTGTCCGACTTGAGGTG-3'
Human glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	5'-ACCACAGTCCATGCCATCAC-3'	5'-TCCACCACCCTGTTGCTGTA-3'