cancers

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*Article*

# Characterization of HMGB1/2 Interactome in Prostate Cancer by Yeast Two Hybrid Approach: Potential Pathobiological Implications

**Aida Barreiro-Alonso [1,†], María Cámara-Quílez [1,†], Martín Salamini-Montemurri [1], Mónica Lamas-Maceiras [1], Ángel Vizoso-Vázquez [1], Esther Rodríguez-Belmonte [1], María Quindós-Varela [2], Olaia Martínez-Iglesias [3], Angélica Figueroa [3] and María-Esperanza Cerdán [1,*]**

**Table S1.** Association of proteins that interact with Hmgb1 or Hmgb2 to cancer hallmarks.

| Cancer Hallmark | Protein | Model | Reference |
|---|---|---|---|
| GENOMIC INSTABILITY AND MUTATIONS/ CHANGES IN TELOMERASE ACTIVITY | MAP1B | Colorectal cancers | [109] |
| | NOP53 | Liver adenocarcinoma (SKHep1) and glioblastoma (T98G) cell lines | [110] |
| | RSF1 | Ovarian cells | [111] |
| | SRSF3 | Ovarian cancer | [112] |
| SUSTAINING PROLIFERATIVE SIGNALLING/ CELL PROLIFERATION | C1QBP | Breast cancer | [54,113] |
| | cFOS | Bladder cancer | [114] |
| | cFOS | Breast cancer | [115] |
| | DLAT | Gastric Cancer | [116] |
| | FLNA | Human melanoma (A7), Prostate cancer (PC3) cell lines | [117] |
| | GOLM1 | Hepatocellular carcinoma | [118] |
| | GOLM1 | Breast cancer | [119] |
| | GOLM1 | Lung proliferation | [120] |
| | GOLM1 | Prostate cancer | [82] |
| | Hox-A10 | Prostate cancer cell line PC-3 | [58] |
| | Hox-A10 | Ovarian cancer  cell lines | [121,122] |
| | NOP53 | Breast cancer | [123] |
| | PSMA7 | Cervical cancer | [124] |
| | PTPN2 | Epithelial carcinogenesis | [125] |
| | RASAL2 | hepatocellular carcinoma | [126] |
| | RSF1 | Prostate cancer | [95] |
| | RSF1 | Nasopharyngeal carcinoma | [127] |
| | SPIN1 | Glioma | [128] |
| | TGM3 | Esophageal cancer | [129] |
| | UBE2E3 | Retinal pigment epithelial (RPE) | [130] |
| | Vigilin | Hepatocellular carcinomas | [131] |
| | WNK4 | Kidney | [100] |
| EVADING GROWTH SUPPRESSORS/ CELL CYCLE CONTROL | C1QBP | Prostate cancer | [48] |
| | COMMD1 | Neuroblastoma | [132] |
| | FLNA | Pulmonary neuroendocrine tumors | [133] |
| | NOP53 | Lung cancer A549  and H1299 cell lines | [107] |
| | RSF1 | Nasopharyngeal carcinoma | [127] |
| | SPIN1 | Glioma | [128] |
| | Vigilin | Liver carcinoma HepG2 cells | [134] |
| RESISTING CELL DEATH APOTOSIS /PHAGOCITOSIS IMBALANCE (continued) | FLNA | bladder carcinoma | [117] |
| | GOLM1 | Prostate cancer | [82] |
| | Hox-A10 | Epithelial ovarian cancer cell lines | [122] |
| | MAP1B | Melanoma and colon carcinoma cell lines | [135] |
| | MAPKAPK5 | Hepatocellular carcinoma | [136] |

| | | | |
|---|---|---|---|
| | MNAT1 | Colorectal cancer | [73] |
| | MT2A | Colorectal cancer | [137] |
| | PSMA7 | Cervical cancer | [124] |
| | PSMA7 | Colorectal cancer | [138] |
| | RSF1 | Nasopharyngeal carcinoma | [127] |
| | SPIN1 | Glioma | [128] |
| | SRSF3 | oral squamous cell carcinoma | [139] |
| | **Protein** | **Model** | **Reference** |
| | SRSF3 | Human colon adenocarcinoma and Human osteosarcoma cell lines | [140] |
| | SRSF3 | Ovarian cancer | [141] |
| | TGM3 | Esophageal cancer | [129] |
| | U2AF1 | Ba/F3 cells<br>Lung adenocarcinoma cell lines | [80,142] |
| | YY1 | Several models | [143] |
| ACTIVATING INVASION AND METASTASIS | C1QBP | Breast cancer metastasis in mouse model | [144] |
| | COMMD1 | Mouse melanoma cells | [145] |
| | FLNA | Human melanoma (A7), prostate cancer (PC3) cell lines | [117] |
| | GOLM1 | Hepatocellular carcinoma | [118] |
| | GOLM1 | Breast cancer | [119] |
| | GOLM1 | Cervical cancer | [146] |
| | GOLM1 | Prostate cancer | [82] |
| | Hox-A10 | Ovarian cancer cell line ES-2 | [121] |
| | MAPKAPK5 | Hela Cells | [86] |
| | MIEN1 | Breast cancer | [147] |
| | MIEN1 | Prostate cancer | [148] |
| | NOP53 | Endometrial cancer | [149] |
| | RASAL2 | Colorectal cancer | [150] |
| | RASAL2 | hepatocellular carcinoma | [126] |
| | RASAL2 | Lung cancer | [151] |
| | RSF1 | Prostate cancer | [95] |
| | RSF1 | Non-small cell lung cancer | [152] |
| | SRSF3 | HeLa cells | [153] |
| | TGM3 | Esophageal cancer | [129] |
| | UHRF2 | Gastric cancer cell lines | [154] |
| | Vigilin | Hepatocellular carcinomas | [131] |
| | YY1 | Cancer cell lines | [155] |
| DEREGULATED CELLULAR ENERGETICS / CARBOHYDRATE METABOLISM REPROGRAMMING | C1QBP | Human embryonic kidney HEK293T cells | [69] |
| | DLAT | Gastric cancer cell lines | [116] |
| | SRSF3 | Colon cancer cells | [156] |

**Table S2.** Prostate cancer studies available through cBioportal.

| cBioportal Name | References | Samples | Sequenced | Cna | RNA Seq | DNA Arrays | Survival Studies |
|---|---|---|---|---|---|---|---|
| Metastatic prostate adenocarcinoma (mctp, nature 2012) | [40] | 61 | 61 | 61 | | | |
| Metastatic prostate adenocarcinoma (su2c/pcf dream team, pnas 2019) | [41] | 444 | 444 | 444 | | | Yes |
| Metastatic prostate cancer (su2c/pcf dream team, cell 2015) | [42] | 150 | 150 | 150 | 118 | | |
| Neuroendocrine prostate cancer (multi-institute, nat med 2016) | [43] | 114 | 114 | 114 | 49 | | |
| Prostate adenocarcinoma (broad/cornell, cell 2013) | [29] | 57 | 57 | 56 | | | |
| Prostate adenocarcinoma (broad/cornell, nat genet 2012) | [30] | 112 | 112 | 109 | | | |
| Prostate adenocarcinoma (cpc-gene, nature 2017) | [31] | 477 | 477 | 0 | | | |
| Prostate adenocarcinoma (eururol, 2017) | [32] | 65 | 65 | 65 | | | |
| Prostate adenocarcinoma (fred hutchinson crc, nat med 2016) | [33] | 176 | 141 | 149 | | | |
| Prostate adenocarcinoma (mskcc, cancer cell 2010) | [34] | 216 | 103 | 194 | | 150 | Yes |
| Prostate adenocarcinoma (mskcc/dfci, nature genetics 2018) | [35] | 1013 | 1013 | 1013 | | | |
| Prostate adenocarcinoma (tcga, cell 2015) | [157] | 333 | 333 | 333 | 290 | | |
| Prostate adenocarcinoma (tcga, pancancer atlas) | [36] | 494 | 494 | 489 | 493 | | |
| Prostate adenocarcinoma (tcga, provisional) | | 499 | 499 | 492 | 498 | | Yes |
| Prostate adenocarcinoma cna study (mskcc, pnas 2014) | [37] | 104 | 0 | 104 | | | Yes |
| Prostate adenocarcinoma organoids (mskcc, cell 2014) | [38] | 12 | 12 | 0 | 10 | | |
| Prostate cancer (mskcc, jco precis oncol 2017) | [39] | 504 | 504 | 504 | | | |
| The metastatic prostate cancer project (provisional, december 2018) | | 19 | 19 | 19 | | | |

**Table S3.** qPCR primer list.

| Gene Name | Sequence | TM (°C) * | Hybridization Site ** | Amplicon Size (bp) |
|---|---|---|---|---|
| ***AGER* (RAGE)** | F: 5′-TGTGTGGCCACCCATTCC-3′ | 60.47 | 901–918 | 109 |
| | R: 5′-CTGATCCTCCCACAGAGCC-3′ | 59.75 | 991–1009 | |
| ***DLAT*** | F: 5′-AACAGCGTGACTACAGGGTATG-3′ | 60.68 | 795–816 | 101 |
| | R: 5′-CCCAAAAGCTGCAGCAGTAAG-3′ | 60.64 | 875–895 | |
| ***FLNA*** | F: 5′-ACAGTGTCAATCGGAGGTCAC-3′ | 60.58 | 4942–4961 | 118 |
| | R: 5′-TGCACGTCACTTTGCCTTTG-3′ | 60.47 | 5040–5059 | |
| ***CFOS*** | F: 5′-GGGATAGCCTCTCTTACTACCAC-3′ | 59.77 | 71–93 | 124 |
| | R: 5′-GTGACCGTGGGAATGAAGTTG-3′ | 60.04 | 174–194 | |
| ***GAPDH*** | F: 5′-CCTCCTGCACCACCAACTG-3′ | 61.18 | 449–467 | 102 |
| | R: 5′-TGGCAGTGATGGCATGGA-3′ | 59.50 | 533–550 | |
| ***HMGB1*** | F: 5′-TCAAAGGAGAACATCCTGGCC-3′ | 60.59 | 338–358 | 87 |
| | R: 5′- GCTTGTCATCTGCAGCAGTGTT-3′ | 62.54 | 403–424 | |
| ***HMGB2*** | F: 5′-GAGCAGTCAGCCAAAGATAAACAA-3′ | 60.37 | 403–426 | 111 |
| | R: 5′-TCCTGCTTCACTTTTGCCCTT-3′ | 61.01 | 493–513 | |
| ***HOXA10*** | F: 5′-CTCCCACACTCGCCATCTC-3′ | 60.43 | 1341–1359 | 187 |
| | R: 5′-CAAACCCAGCCCAGTCAGG-3′ | 61.19 | 1509–1527 | |
| ***KLK3* (PSA)** | F: 5′-ACCCTGGCAGGTGCTTG-3′ | 60.08 | 108–124 | 116 |
| | R: 5′-GCAAGATCACGCTTTTGTTCCT-3′ | 60.61 | 202–223 | |
| ***KRT7*** | F: 5′-TGAATGATGAGATCAACTTCCTCAG-3′ | 59.25 | 653–677 | 75 |
| | R: 5′-TGTCGGAGATCTGGGACTGC-3′ | 61.91 | 708–727 | |
| ***MAP1B*** | F: 5′-ACATCTTGGAACCTCCCACATC-3′ | 60.62 | 731–752 | 98 |
| | R: 5′-TGCAAACAAGGCAGAATCGC-3′ | 60.61 | 809–828 | |
| ***MIEN1*** | F: 5′-TTGGGGGCAGGAGAGAGAC-3′ | 61.21 | 519–537 | 107 |
| | R: 5′-TTACCGAGGCGAAGAGTGG-3′ | 59.68 | 607–625 | |
| ***MNAT1*** | F: 5′-TGTGCGGACACACTCTCTGTGAAA-3′ | 65.09 | 74–97 | 145 |
| | R: 5′-TCAACCTCCTTGTCAACAGTGGGA-3′ | 64.37 | 195–218 | |
| ***MT2A*** | F: 5′-AAAGGGGCGTCGGACAAG-3′ | 60.54 | 223–240 | 118 |
| | R: 5′- GGTCACGGTCAGGGTTGTAC-3′ | 60.90 | 321–340 | |
| ***NOP53*** | F: 5′-ACCAGTTCCTGGAAGACGTG-3′ | 60.19 | 182–201 | 109 |
| | R: 5′-CCTTTTTCCTTGGAGCCAG-3′ | 56.66 | 272–290 | |
| ***PMEPA1*** | F: 5′-AAGAGGAGTGAGAGGAAGGC-3′ | 59.02 | 929–948 | 110 |
| | R: 5′-GCTTGTGCATTCAGACCAGA-3′ | 59.05 | 1019–1038 | |
| ***SNAPIN*** | F: 5′-TGACAACCTAGCCACAGAACTG-3′ | 60.81 | 174–195 | 97 |
| | R: 5′-TCGCCGGGCATTAAGTAGC-3′ | 60.80 | 252–270 | |
| ***UBE2E3*** | F: 5′-AAGGTTACTTTCCGCACCAG-3′ | 58.69 | 376–395 | 102 |
| | R: 5′-AATAGTCAAAGCGGGACTCCA-3′ | 59.69 | 457–477 | |
| ***UHRF2*** | F: 5′-GGACCTTCCAATCAGCCATC-3′ | 58.90 | 316–335 | 99 |
| | R: 5′-GACATCTCTGGCATCCACCA-3′ | 60.04 | 395–414 | |
| ***YY1*** | F: 5′-AGCGGCAAGAAGAGTTACCTC-3′ | 60.65 | 538–558 | 103 |
| | R: 5′-TCTTGATCTGCACCTGCTTCTG-3′ | 61.20 | 619–640 | |
| ***ZNF428*** | F: 5′-CCCGAGCATTCCTCTGATTC-3′ | 58.70 | 79–98 | 95 |
| | R: 5′-TCGTCAGTGGTCTCCTCTTC-3′ | 59.04 | 154–173 | |

* Tms were calculated using MFEprimer3.0 (http://mfeprimer.igenetech.com/) ** Hybridization sites are referenced to the starting codon (cDNA) for each gene (Ensembl database).

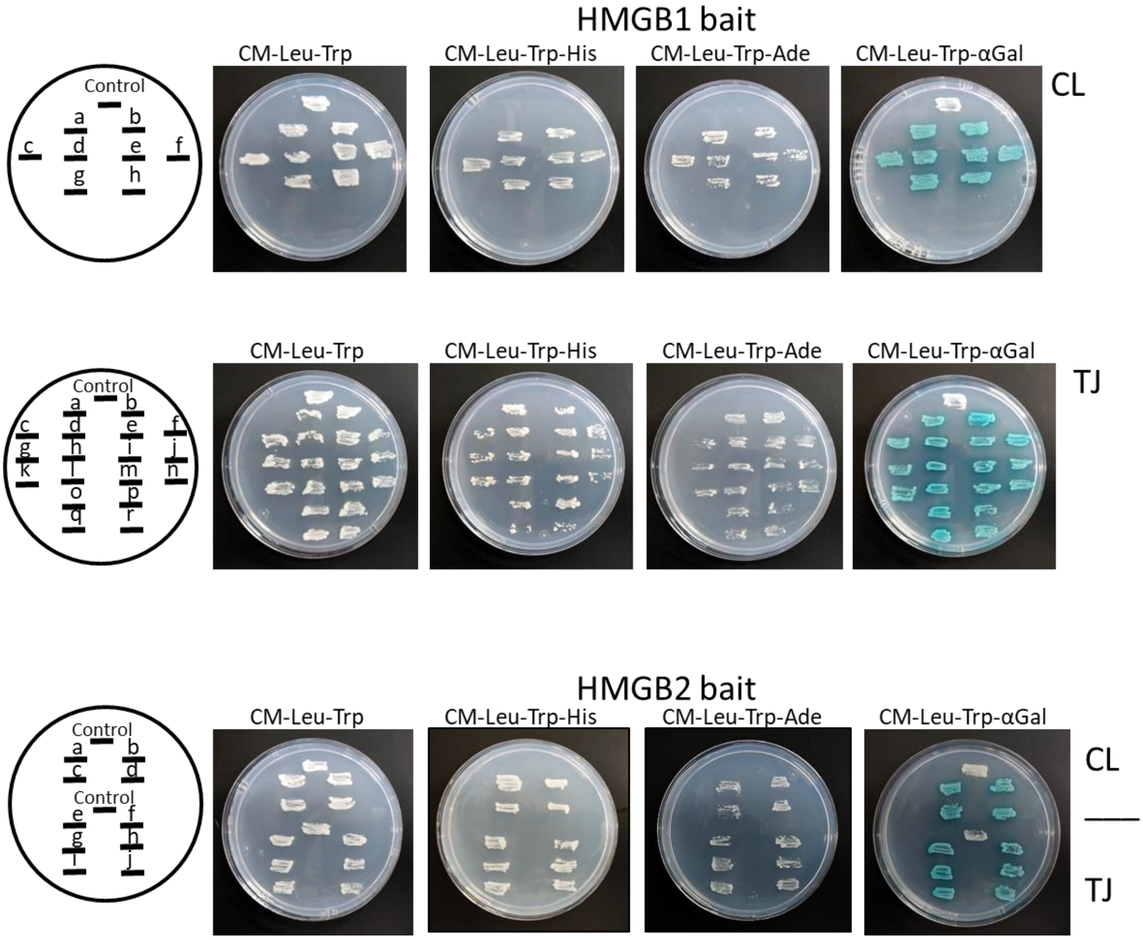
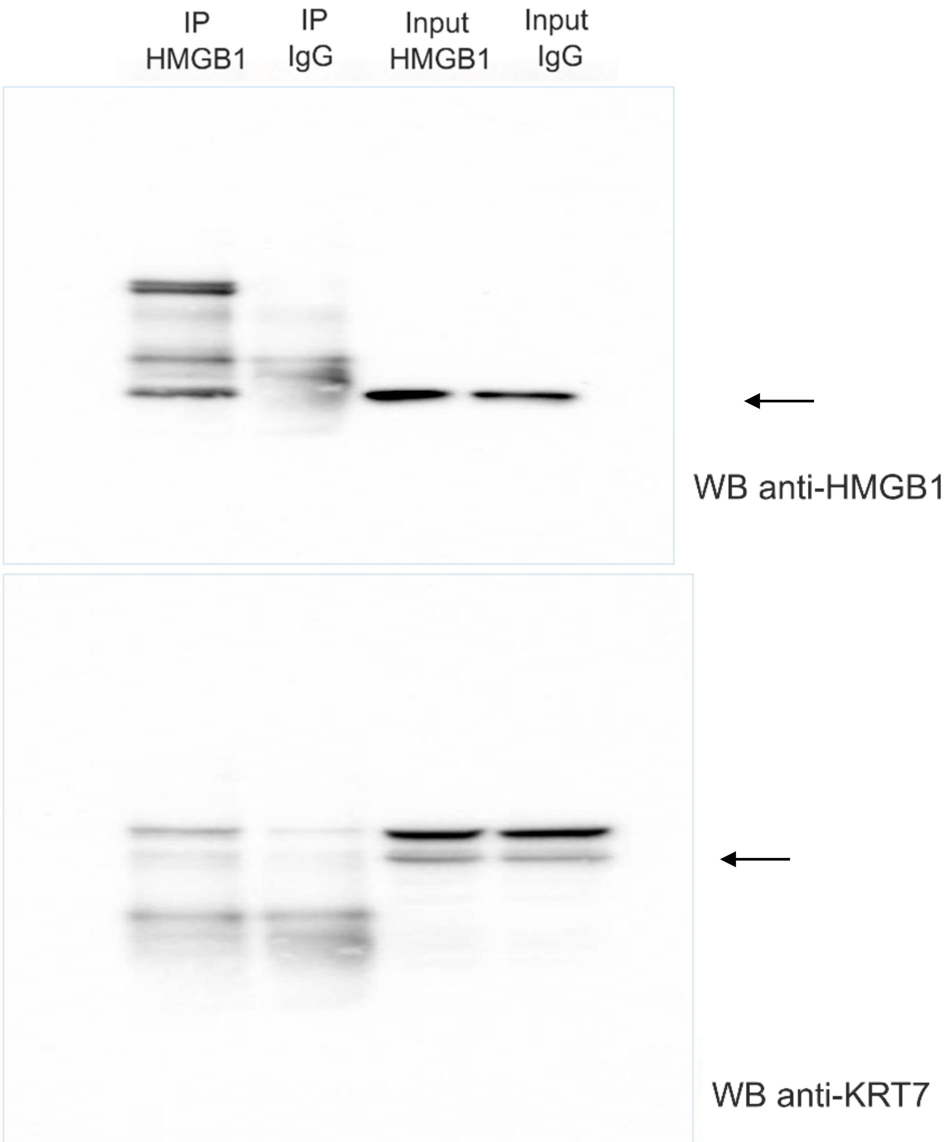



**Figure S1.** Re-screening of Y2H candidates and triple selection for positive growth in media without histidine, adenine or reporting alfa-galactosidase activity. Single colonies which were positive in the first screening as described in Materials and Methods were re-plated on selective media. CL, candidates from PC-3 libraries; TJ, candidates from PCa tissue libraries. Control represent cells transformed with the corresponding bait plasmid and an empty prey-plasmid. Upper panel: a, DLAT; b, DANNAF; c, HDLBP; d, HOXA10; e, KRT7; f, UBE2E3; g, UHRF2; h, ZNF428. Middle panel: a, FOS; b, GOLM1; c, HNRHPU; d, MAP1B; e, MAPKAPK5; f, MIEN1; g, MT2A; h, PSMA7; i, PTPN2; j, RASAL2; k, RSF1; l, SRSF3; m, TAF3; n, TGM3; o, UBC; p, WINK4; q, YY1; r, ZNF48. Lower panel: a, C1QBP; b, SNAPIN; c, UHRF2; d, ZNF428; e, C1QBP; f, COMMD1; g, FLNA; h, MIEN1; i, NOP53; j, RSP28.

**Image S1.** Whole Blots accompanying Figure 2A



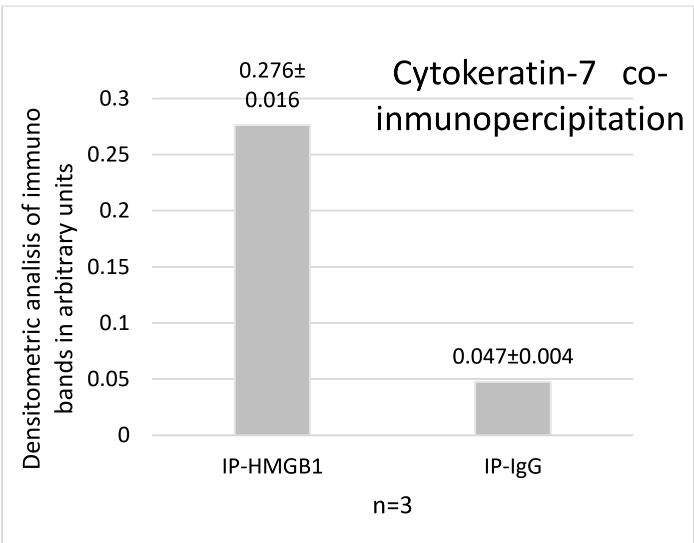
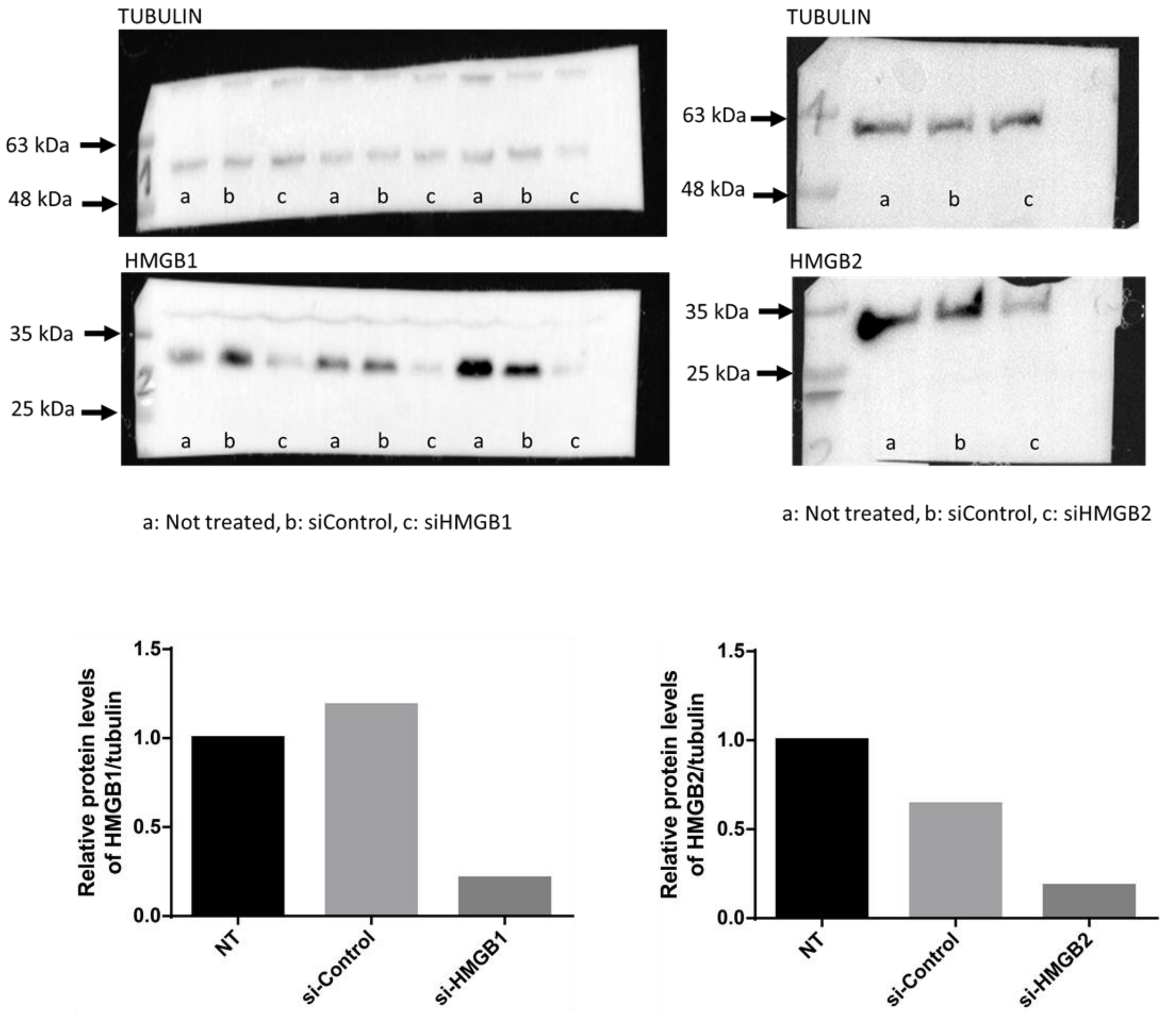



**Image S2.** Whole Blots accompanying Figure 6.



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