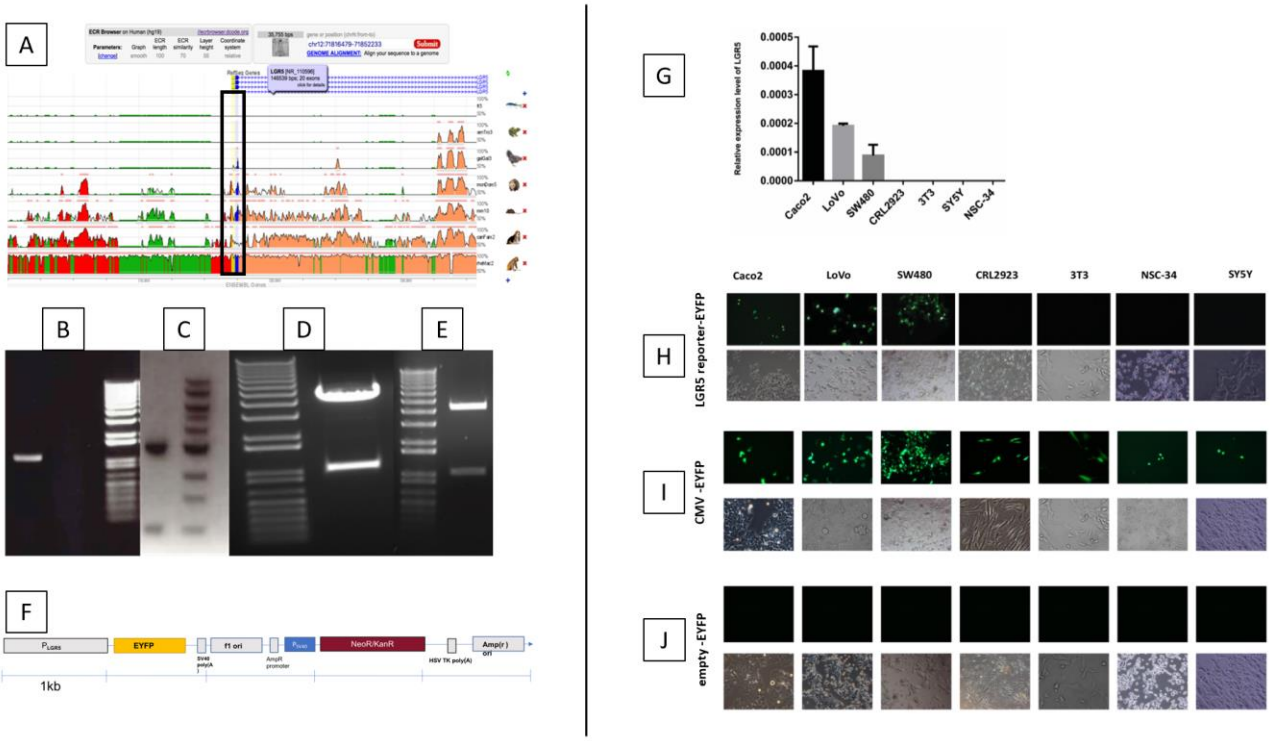
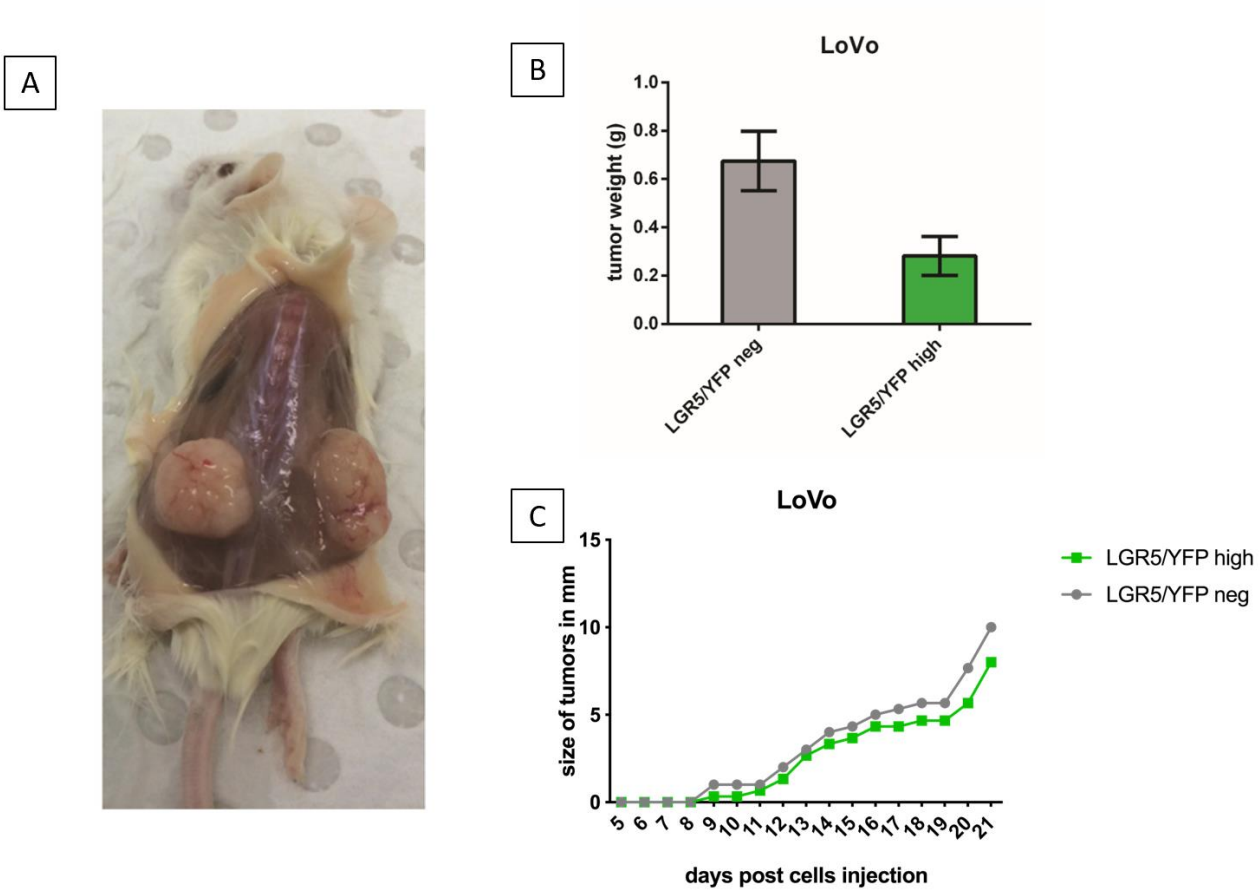
Supplementary material



Supplementary Figure 1 Generation and validation of LGR5 promoter-based reporter. **A.** Snapshot of the ECR browser showing the conserved regions of LGR5 promoter (grey Square). **B.** The LGR5 promoter fragment amplified from RP11-59F15 bacterial artificial chromosome (BAC) clones. **C.** Amplification from BAC; Digestion of promoter-pGEM-T Easy plasmid with HindIII and NdeI showing vector band of 3kb and promoter band of 1kb; **D.** Digested promoter-pEYFP-N1 clone with HindIII and NdeI showing 1kb promoter band and 4kb vector band; **E.** Digestion of promoter-pEYFP-N1 clone for negative-control clone; promoter-pEYFP-N1 clone digested with XhoI and SalI to be self-ligated as a negative-control clone. **F.** Plasmid map of the pLGR5/EYFP reporter. **G.** *LGR5* mRNA expression in 7 different cell lines. *LGR5* mRNA expression was evaluated by qRT-PCR analysis, and normalized to *GAPDH*. *LGR5* mRNA was frequently overexpressed in colon cancer cell lines (Caco-2, LoVo and SW480) but not expressed in the other cell lines investigated. **H.** Transient transfection analysis of expression of the EYFP reporter. Caco-2, LoVo and SW480, endogenously express LGR5 and thus show EYFP reporter expression after transfection with the LGR5 promoter-reporters; in contrast no EYFP expression is observed in transfected human dermal fibroblast (CRL2923), mouse fibroblast (3T3), human neuroblastoma cell line (SH-SY5Y) or mouse motor neuron cell line (NSC-34). **I.** The same cell lines transfected with the pEYFP-N1 (Clontech) vector (CMV driven EYFP vector; positive control) show wide spread expression, with expression levels most likely reflecting the cell line-specific efficiency of the transfection. **J.** The same cell lines transfected with the negative control (empty-EYFP or promoter-less) construct show no EYFP expression.



***Supplementary Figure 2 In-vivo tumorigenic assay of LoVo colon cancer* cell line.** Sorted LGR5/EYFPneg and high cells were injected subcutaneously in left and right flanks (respectively) of NOD-SCID mice and monitored for 3 weeks. **A.** Representative images of tumours formed by LGR5/EYFPhigh (right flank) and LGR5/EYFPneg (left flank) cells. Tumour weights (**B.)** and size (**C.)** resulting from LGR5/EYFPneg and LGR5/EYFPhigh sorted cells. Columns and error bars represent means ± SD of two independent experiments using duplicate measurements for each experiment.