**Supplementary Methods**

**Whole Exome Sequencing (WES)**

DNA from 12 fresh frozen tumor samples (five pure SEF, two hybrid SEF/LGFMS and five LGFMS) was used for WES. Accompanying peripheral blood samples were available for the LGFMS tumors, but not for the SEF or hybrid SEF/LGFMS tumors. DNA extraction was performed as described (26). Whole exome capture, template preparation, and sequencing on the Ion Chef and Ion Proton systems, using the Ion PI IC 200 kit (ThermoFisher Scientific, Waltham, MA, USA), were performed as described (40). Variant calling for LGFMS tumors with accompanying blood samples was performed with the AmpliSeq Exome tumor-normal pair work-flow in Ion Reporter (ThermoFisher Scientific), as well as with the Torrent Variant Caller plugin in the Torrent Suite Software (ThermoFisher Scientific), using standard parameters for somatic variant detection. Variant calling for SEF and hybrid SEF/LGFMS tumors without accompanying normal constitutional DNA was performed with the Torrent Variant Caller plugin in the Torrent Suite Software (ThermoFisher Scientific). Annotation of detected variants was performed in Ion Reporter, using the GRCh37/hg19 assembly. The results were further filtered as follows: mutations with less than 5 supporting reads, copy number variants, mutations outside exons, single nucleotide variants reported in the 5000 Exome project, synonymous exonic mutations, identical mutations detected in 5 OFMTs analyzed simultaneously, and mutations that were not included in the COSMIC database were all discarded.

125 mutations detected at WES on SEF and SEF/LGFMS were verified using a TruSeq Custom Amplicon (TSCA) panel (Illumina). The TSCA covered the regions of thegenes harboring mutations detected at WES. DNA was processed and analyzed as described for WES analysis.

In addition, DNA from two FFPE samples from a hybrid SEF/LGFMS – one showing LGFMS morphology (17A) and one SEF morphology (17B) – was sent to GATC Biotech (Konstanz, Germany) for WES. DNA was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen), according to the manufacturer’s instructions. In brief, using 500 ng DNA as the starting material, human all exon enrichment was obtained with Agilent SureSelectXT (Agilent) and paired-end 125 bp sequencing was done on an Illumina HiSeq (Illumina) at x90 coverage. Variant calling was performed using the MuTect algorithm (41); the two samples were used as controls for each other. The results were filtered by keeping only non-synonymous exonic mutations that were not reported in the dbSNP database.

**Tet-On 3G setup**

Transductions were performed by RetroNectin-bound virus infection method according to the manufacturer’s instructions (Takara/Clontech). Tet regulator transduced cells were selected for by addition of the G418 antibiotic to the culture medium at a final concentration of 0.1mg/ml and response plasmid transduced cells by addition of puromycin to a final concentration of 2µg/ml. After each of the two transduction steps, expression of the regulator protein gene or the introduced fusion gene was controlled for by quantitative real-time PCR (qRT-PCR).

Fusion gene expressing cells were cultured with or without doxycycline (dox) for 96 hrs. They were subsequently pelleted in RLT buffer (Qiagen, Valencia, CA, USA) with 1% mercaptoethanol and RNA was extracted using RNeasy micro kit (Qiagen) according to the manufacturer’s instructions.

**Constructing the pLVX-TRE3G response plasmids**

The full-length coding sequence of *FUS-CREB3L2* from Case 25 (bp 727-bp 1073) cloned into pCR3.1 (42) was used as a template in the subcloning procedure of the pLVX-TRE3G-FUS-CREB3L2 vector, which was constructed with the In-Fusion HD Cloning Kit (Clontech), according to the manufacturer’s instructions.

The pLVX-TRE3G-EWSR1-CREB3L1 and pLVX-TRE3G-EWSR1-NR4A3 constructs were synthesized by Genscript (Piscataway, NJ, USA). The fusion transcripts were based on sequencing findings: *EWSR1-CREB3L1*, bp 1302 (ex 8) - 6bp insert- bp 1206 (part of ex 6) and *EWSR1-NR4A3*, bp 1622 (ex 12) - bp 751 (part of ex 3).

**qRT-PCR**

To evaluate the expression of the introduced Tet-On regulator and the different fusion genes in the Bj5Ta cell line, TaqMan gene-expression assays were performed with custom-made probes for *EWSR1-CREB3L1*, *FUS-CREB3L2*, *EWSR1-NR4A3* and the Tet-On 3G construct. The *TBP* gene was used as endogenous control. qRT-PCR was performed according to the manufacturer's instructions, and all reactions were run in triplicate (Applied Biosystems). Calculations were done using ΔΔCt method using the SDS software 1.3.1 (Applied Biosystems).

**Flow cytometry**

For cell surface CD24 staining, cells in PBS + 2% FBS were incubated with a PE-conjugated mouse anti-human CD24 antibody (BD biosciences, San Jose, CA) for 20 min in a cold room. Following washing, cells were analyzed in an LSR Fortessa cytometer (BD biosciences). To investigate the total CD24 expression, the cells were permeabilized with 1.6 % formamide for 10 min at room temperature, spun down for 3 min at 2800 rpm, resuspended in ice cold ethanol and washed 2x in PBS + 2% FBS. Antibody staining and FACS analysis were then performed as above.

**References**

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